REMARKS

Claims 1, 5-7 and 10 are pending in the application, Claim 11 has been deemed withdrawn by the Examiner. For reasons as set forth below, Applicants submit that reconsideration and allowance of the claims is appropriate.

35 U.S.C. §103 Claim Rejection

Claim 1, 5-7 and 10 are rejected under 35 U.S.C. 103(a) by the Examiner as being allegedly unpatentable over Kong et al. This rejection is respectfully traversed for the reasons as stated below.

As correctly quoted by the Examiner, the Applicant respectfully submits that Kong et al. do not disclose how to make unbranched polysaccharides and that making such unbranched polysaccharides would be beyond the ordinary level of skill in the art.

To the contrary, according to the Examiner, "*making an unbranched oligosaccharide would, at the very most, require merely a routine modification of the protocol for synthesizing a branched oligosaccharide as disclosed by Kong et al.*". To support this statement, the Examiner proposes a modification of the synthetic scheme disclosed in Kong et al. wherein the Examiner has erased the branched saccharides. The Examiner concludes that "*these modifications are clearly in the ordinary level of skill in the art*".

The argument of the Examiner cannot be followed for the following reasons.

1) As mentioned by Richard Schmidt (in Zhu et al., "New principles for glycoside-bond formation," *Angew. Chem. Int. Ed.* 2009, 48, 1900-1934, copy attached), who is an emeritus professor in sugar chemistry and world-wide recognized as an eminent specialist, "*many of the steps required in glycoside synthesis involve the selective protection and deprotection of hydroxyl groups (and sometimes amino groups). Therefore, protecting-group manipulation often takes up the most time in glycoside*

synthesis." (see p.1914, §3.1.). In other words, the synthesis of glycosides is complex and difficult, and requires a step-by-step approach different for each glycoside compound. Consequently, the synthesis of unbranched oligo-β-(1,3)-glucans according to the invention requires more than a simple erasing of the branched saccharides in the synthesis scheme disclosed in Kong et al. In this regard, the oligo-β-(1,3)-glucans according to the invention have been synthesized in an original way, totally different from the synthesis proposed by Kong et al., and which is fully described in the description (see for instance example 2, wherein an original protecting group 2-methylnaphtyl (NAP) is used).

2) <u>Kong himself</u>, in Ning et al., *Bioorganic & Medicinal Chemistry* 11 (2003) 2193-2203 (copy attached), shows that the synthesis of glycosides is complex. For instance, when synthesizing <u>branched</u> oligo-β-(1,3)-glucans, Kong shows that, depending on the nature of the compounds which are put in reaction, it is not always possible to obtain the coupling of the saccharides (see for instance scheme 3, wherein compounds 29+23 do not lead to any coupling product). In addition, Kong clearly shows that, depending on the experimental conditions which are used, α or β bonds are obtained in an unpredictable way: "*It is interesting to find that coupling of a 3,6-branched acylated trisaccharide trichloroacetimidate donor **9** with **3,6**-branched acceptors **13** and **16** with **3'-OH** gave the <u>α-(1-3)-linked</u> hexasaccharides **17** and **19**, respectively, <u>in spite of</u> the presence of C-2 ester capable of neighboring group participation*" (see abstract, line 3-5). In other words, Kong himself shows that it is not possible to predict the synthesis of glycosides.

3) Again, <u>Kong himself</u>, in Zeng et al., *Tetrahedron Letters* 43 (2002) 3729-3733 (copy attached), shows that the synthesis proposed by the Examiner does not work in experimental conditions. Indeed, as explained by Kong in page 3730 (across first and second paragraph, see also fig.1), "*Condensation of a (1,3)-β-linked disaccharide donor **18** with a trisaccharide acceptor **19** having a (1,3)-β-linkage at the non-reducing end yielded sole <u>α-linked</u> pentasaccharide **21***". In other words, the synthesis proposed by the Examiner does not lead a linear oligo-β-(1,3)-

pentaglucan (i.e. which comprises only β bonds) but to a β/α/β/α tetrasaccharide, which is therefore different from the laminaripentaose according to the invention. In the same spirit, in fig.1 p.3731, when Kong couples two β-disaccharides (compounds 14 and 15) together, a β/α/β tetrasaccharide is surprisingly obtained, which is therefore different from the laminaritetraose according to the invention. The results of Kong therefore clearly confirm that the synthesis of glycosides is unpredictable.

The statement of the Examiner according to which "*these modifications* [of the synthesis scheme of Kong et al.] *are clearly in the ordinary level of skill in the art*" is thus incorrect, as shown by Kong himself.

Consequently, the Kong et al. reference is not considered enabled for the unbranched oligosaccharides.

It is thus the case that the teaching of Kong et al. cannot serve as basis for an obviousness rejection: the skilled person, having knowledge of Kong et al. and wanting to test the activity of unbranched oligosaccharides would never have succeeded since he would never have obtained these oligosaccharides...

The claimed invention is thus nonobvious in view of Kong et al. for at least this reason.

Second, and as previously mentioned by the Applicant, Kong et al. do not provide any experimental data supporting the alleged anti-tumor activity of unbranched oligosaccharides (see applicant's arguments presented in response to the office action dated April 15, 2009). The only suggestion of Kong et al. is that a branched oligosaccharide extracted from the fungus *Lentinus edodes* might have an anti-tumor activity. This teaching correlates with the results disclosed in the Ning et al. reference, previously cited by the Examiner.

However, according to the Examiner, "*all that is needed is a reasonable expectation of success for practicing the claimed invention based on Applicant's disclosure and the ordinary level of skill in the art*". In this regard, the Applicant respectfully submits an article published by one of the co-inventors, Dr. Vaclav Vetvicka of the University of Louisville (Vetvicka et al., *JANA*, Vol.10, No.1, 2007, copy attached). In this article concerning the evaluation of the immunological activities of commercially available β-(1,3)-glucans, it is concluded that :

- "*... with the high number of individual glucans and huge differences in their biological activities, it is imperative to evaluate their biological properties before any suggestions for use of a particular glucan can be made*" (see page 29, end of first paragraph, beginning of second paragraph), and

- "*Also, it is clear that individual glucans can be highly active in one particular part of immune reactions [...] and almost without any significant biological activity in other parts of defense reaction*" (see p.30, right column, third paragraph).

Accordingly, in the technical field of sugars, it is <u>impossible to predict</u> the biological activity of a particular sugar by comparing it to already known sugars.

In conclusion, the Kong et al. reference discloses:

- neither a method leading to the synthesis of laminaritetraose and laminaripentaose,

- nor an anti-tumor activity of laminaritetraose and laminaripentaose.

The sole teaching of Kong et al. is that a particular <u>branched</u> oligosaccharide may have anti-tumor activity (but no evidence is provided), which correlates with the teaching of the Ning et al. reference, already cited by the Examiner.

Accordingly, Kong et al. do not provide any supplementary information compared to Ning et al., and the skilled person, having knowledge of Kong et al. in view of Ning et al. would thus have considered that only the branched oligosaccharides might have anti-tumor activity.

Consequently, since neither the two particular oligo-β-(1,3)-glucans of the claimed invention nor their anti-tumor activity are disclosed, taught or suggested in Kong et al., claim 1 is non obvious.

Since claim 1 is non obvious, claims 5-7 and 10, which depend on claim 1, are also non obvious.

In view of the above arguments, it is considered that the claims are patentable over the cited Kong et al, reference, and that the application is now in proper form for allowance.

Consideration of these arguments and prompt allowance of the above claims are thus respectfully requested.

Respectfully submitted,

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By: B. Aaron Schulman
Registration No.: 31,877

STITES & HARBISON PLLC◆1199 North Fairfax St◆Suite 900◆Alexandria, VA 22314
TEL: 703-739-4900 ◆ FAX: 703-739-9577 ◆ CUSTOMER NO. 000881

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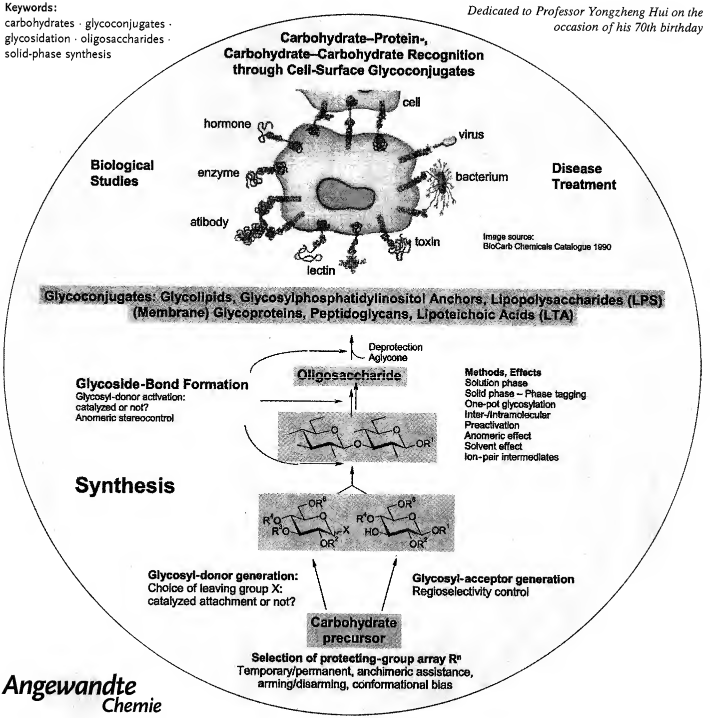
New Principles for Glycoside-Bond Formation

Xiangming Zhu and Richard R. Schmidt**

Keywords:
carbohydrates · glycoconjugates · glycosidation · oligosaccharides · solid-phase synthesis

Dedicated to Professor Yongzheng Hui on the occasion of his 70th birthday



Angewandte Chemie

Increased understanding of the important roles that oligosaccharides and glycoconjugates play in biological processes has led to a demand for significant amounts of these materials for biological, medicinal, and pharmacological studies. Therefore, tremendous effort has been made to develop new procedures for the synthesis of glycosides, whereby the main focus is often the formation of the glycosidic bonds. Accordingly, quite a few review articles have been published over the past few years on glycoside synthesis; however, most are confined to either a specific type of glycoside or a specific strategy for glycoside synthesis. In this Review, new principles for the formation of glycoside bonds are discussed. Developments, mainly in the last ten years, that have led to significant advances in oligosaccharide and glycoconjugate synthesis have been compiled and are evaluated.

From the Contents

1. Introduction

Most carbohydrates found in nature exist as polysaccharides, glycoconjugates, or glycosides, in which sugar units are attached to one another or to aglycones through O-glycosidic bonds. Thus, the stereoselective formation of O-glycosidic bonds is the key process in most glycoside syntheses. Since the first glycoside syntheses by Michael[1] and Fischer,[2] followed by the seminal studies of Koenigs and Knorr,[3] a very large number of glycosidation methods have been developed. In this Review, advances in the formation of O-glycoside bonds are examined, with emphasis placed on developments in the last ten years. A detailed discussion of new glycosidation methods is preceded by an overview of the general principles for the formation of glycoside bonds.

The chemical synthesis of glycosides usually involves the transformation of a sugar into a fully protected glycosyl donor with a leaving group at its anomeric center. Glycosylation of a suitably protected glycosyl acceptor, which generally contains only one free hydroxy group, then follows. (In other words, the "glycosyl donor" transfers the glycosyl moiety (generally as an electrophile) to the "glycosyl acceptor" (generally the nucleophile)).[4–6] Hence, the leaving group of the glycosyl donor and the protecting groups are the most fundamental parameters with respect to the yield and anomeric selectivity of glycosidation reactions (as outlined in Sections 2 and 3).

Often used methods for the generation of glycosyl donors are oxygen-exchange reactions at the anomeric position of the hemiacetal moiety of pyranoses and furanoses.[4a,7,8] The Fischer–Helferich method (Figure 1, **A**), an acid-catalyzed reaction for the direct replacement of the anomeric oxygen atom, has been applied successfully to the synthesis of many glycosylation substrates. However, the reversibility of the reaction limits its usefulness in the synthesis of complex oligosaccharides and glycoconjugates. For irreversible exchange of the anomeric oxygen atom, preactivation of the anomeric center through the introduction of a good leaving group is necessary.

The best known of these irreversible methods is the Koenigs–Knorr method (Figure 1, **B**), in which an α-halo ether is generated as the glycosyl donor (see Section 2.1). This intermediate is further activated by halophilic promoters in the glycosylation step. Generally, between one and four equivalents of the promoter (for this reason, the term "catalyst" should not be used) and often additional reagents (for example, a sterically hindered base) are used in the reaction, which results in an irreversible transfer of the glycosyl moiety to the acceptor. The obvious limitations of this method prompted the search for alternative methods.[4a,5,7–10]

Other approaches closely related to the Koenigs–Knorr method have been investigated extensively. The exchange of the anomeric oxygen atom for a fluorine, alkylthio, or arylthio leaving group found great interest, as these groups are not affected by manipulations of orthogonal protecting groups (see Section 2.2). Also, one-pot consecutive glycosylation reactions of acceptors are possible (new developments are discussed in Sections 2.1–2.6). However, the advantages and the fundamental drawbacks of the Koenigs–Knorr method are also associated with these activation systems.

In the methods described above, the anomeric carbon atom of the sugar residue to be coupled serves as the electrophile and the alcohol as the nucleophile. A useful alternative would be the base-mediated deprotonation of the anomeric hydroxy group of a pyranose or furanose moiety to generate an anomeric oxide, which would undergo direct and

[*] Dr. X. Zhu
Centre for Synthesis and Chemical Biology, UCD School of Chemistry and Chemical Biology, University College Dublin
Belfield, Dublin 4 (Ireland)
Fax: (+353) 1-716-2501
E-mail: xiangming.zhu@ucd.ie
Prof. Dr. R. R. Schmidt
Fachbereich Chemie, Universität Konstanz, Fach 725
78457 Konstanz (Germany)
Fax: (+49) 7531-883-135
E-mail: richard.schmidt@uni-konstanz.de

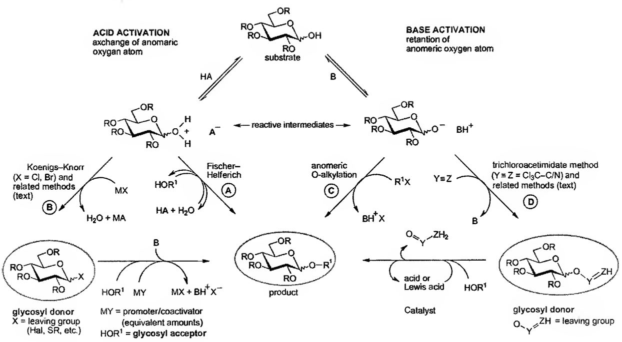


Figure 1. Generation of glycosidic and saccharide bonds.

irreversible anomeric *O*-alkylation to give a glycoside (Figure 1, **C**). Surprisingly, this simple "anomeric *O*-alkylation" method, as termed by us,[4a,7,8] had not been used for the synthesis of complex glycosides and glycoconjugates prior to our own studies. The direct anomeric *O*-alkylation of variously protected and even totally unprotected sugars in the presence of a base with triflates or Michael acceptors as alkylating agents has become a very convenient method for glycoside-bond formation.[11–14] The high anomeric stereoselectivity that is often observed with pyranoses results from the enhanced nucleophilicity of equatorial oxygen atoms (owing to steric effects and the stereoelectronic kinetic anomeric effect due to repulsions of lone electron pairs, dipole effects, or both)[4a,7,8] and from the higher stability of products with an axial anomeric oxygen atom (owing to the thermodynamic anomeric effect due to $n\sigma^*$-orbital interactions, favorable dipole effects, or both). Chelation effects can also be used to promote anomeric stereoselectivity. The availability and to some extent the stability of the carbohydrate-derived alkylating agents preclude the general applicability of this simple method to the synthesis of complex oligosaccharides and glycoconjugates.

There are three main requirements for an efficient glycosylation method:

- Small amounts of the reagents must be used; that is, the glycosyl donor must be generated in a simple process and the donor activated by a catalytic amount of a reagent;
- the glycosylation step must be stereoselective and high-yielding;
- the method must be applicable on a large scale.

These demands are not met by any of the methods described above. However, the general strategy for glycoside-bond formation is reasonable: The first step (generation of the glycosyl donor) should consist of the preactivation of the anomeric center with the formation of a stable glycosyl donor, ideally through a catalytic reaction to attach a leaving group to the anomeric hydroxy group. The second step (activation of the glycosyl donor) should consist of a sterically uniform high-yielding glycosyl transfer to the glycosyl acceptor on the basis

Xiangming Zhu received his PhD in organic chemistry from the Shanghai Institute of Organic Chemistry in 2001. After a period as a postdoctoral fellow at the University of Konstanz with Professor Richard R. Schmidt, he became an Associate Professor at Zhejiang University. In 2005, he spent one year in the research group of Professor Geert-Jan Boons at the Complex Carbohydrate Research Center in the USA, where he studied the synthesis of arabinofuranosides. In 2006, he moved to the University College Dublin as a lecturer.

Richard R. Schmidt completed his PhD at the University of Stuttgart in 1962 under the guidance of Professor Rudolf Gompper on push–pull-stabilized quinone methides. From 1965 to 1966, he held a postdoctoral fellowship with Professor Frank M. Huennekens at the Scripps Research Foundation in La Jolla, USA, where he investigated the metabolism of coenzyme B12. He was Full Professor at the University of Konstanz from 1975 to 2003 and is now Emeritus Professor. In recent years, his research has mainly focused on glycoconjugate chemistry and its biological relevance.

of activation of the glycosyl donor with a catalytic amount of a promoter (that is, a catalyst) and covalent binding of water released in this condensation reaction to the leaving group. In this way, the required amounts of reagents can be minimized.

Experience with direct anomeric O-alkylation showed that these demands can essentially be fulfilled with a simple base-catalyzed transformation of the anomeric oxygen atom into a leaving group and the acid-catalyzed activation of this group in the glycosylation step. These orthogonal activation and glycosylation steps should also satisfy the demand for simplicity in combination with efficiency, which are critical for general acceptance.

Electron-deficient nitriles, such as trichloroacetonitrile (Figure 1, **D**: X≡Y = $CCl_3C{\equiv}N$), undergo direct and reversible base-catalyzed addition of the anomeric hydroxy group to provide *O*-glycosyl trichloroacetimidates. The bulky and strongly electron withdrawing trichloromethyl group, and the glycosyl group, which facilitates the formation of an oxocarbenium ion at the anomeric center through the α oxygen atom, provide the driving force for the acid-catalyzed release of trichloroacetamide as the leaving group. Trichloroacetamide does not exhibit acid or base properties under the reaction conditions, which makes acid catalysis possible. Hence, upon acid-catalyzed activation, *O*-glycosyl trichloroacetimidates exhibit excellent glycosyl-donor properties (see Section 2.7).

Closely related methods are the activation of the anomeric hydroxy group by trifluoroacetonitrile, dichloromalonitrile, and dichloroacetonitrile.[15–18] Ketenimines, which undergo addition of the anomeric hydroxy group under base catalysis, provide another important class of glycosyl donors. However, as only a few examples have been investigated to date, the potential of these glycosyl donors has not yet been established.[15,19,20] Another interesting class of compounds is that of imide halides with electron-withdrawing carbon substituents and their heterocyclic equivalents. Following some earlier studies,[15,21–25] imide halides have recently found increased interest and been used glycosylation reactions with excellent results (see Section 2.7).

Other related methods include the activation of the anomeric hydroxy group, for example, through sulfate, sulfonate, phosphate, or phosphite formation, as described in Section 2.8 for *O*-glycosyl phosphates. However, beside the drawbacks associated with activation through the formation of an imide halide, a further disadvantage is the increase in the acidity of the reaction mixture in the glycosylation step upon the release of these leaving groups.

Glycals, which are readily available from sugars, are also attractive substrates for the formation of glycoside bonds (Figure 2). Their nucleophilicity at C2 enables reactions, for example, with oxygen, nitrogen, and sulfur electrophiles, to be carried out with high substrate stereoselectivity, generally with the formation of a three-membered ring; ring opening with alcohols as acceptors under acid catalysis, either directly by method **Ea** or by method **Eb** with Y as a promoter, furnishes the corresponding glycosides.[26–33] With an appropriate electrophile X, this method can also be employed for 2-deoxyglycoside synthesis.

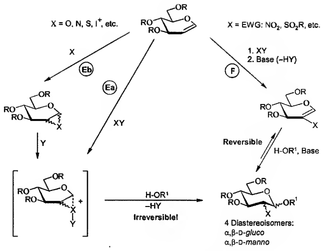


Figure 2. Glycals as intermediates for the generation of glycosidic bonds.

Glycals can also be transformed into derivatives with an electron-withdrawing group at C2, for example, into 2-nitroglycals, which may undergo Michael addition. Thus, glycoside-bond formation under base catalysis (method **F**) leads to 2-deoxy-2-nitroglycosides.[34] These intermediates are readily converted into 2-amino-2-deoxyglycosides, which are constituents of almost all glycoconjugates. Recently, this 2-nitroglycal concatenation was investigated extensively, in particular with 2-nitrogalactal derivatives (see Section 2.9).

Besides substrates and leaving groups, promoters also have a significant influence on glycosidation selectivity by affecting the formation of reaction intermediates. Therefore, careful optimization of the promoter in accord with the reaction partners is crucial for stereoselective glycoside-bond formation;[4–6] it is sometimes quite a challenge to find a promoter system that leads to high stereoselectivity and a high yield in a particular glycosidation. The promoter system is also very important with respect to performing the glycosidation reaction on an industrial scale. In this regard, *O*-glycosyl trichloroacetimidates[19] have great advantages. They are among the most widely used glycosyl donors in contemporary carbohydrate chemistry.[4a]

Glycoside-bond formation often leads to a mixture of two anomeric stereoisomers, that is, 1,2-*cis* and 1,2-*trans* glycosides. Neighboring-group participation in 2-*O*- or 2-*N*-acyl-protected glycosyl donors or glycosyl donors with sterically demanding protecting groups at the 2-position leads reliably to 1,2-*trans* glycosides. Accordingly, the presence of a sterically nondemanding, nonparticipating group at the 2-position is often used for the synthesis of 1,2-*cis* glycosides. However, the effect of the presence of nonparticipating groups is often insufficient to guarantee stereoselective *cis* glycosylation reactions because most glycosidation reactions proceed by an S_N1 mechanism via oxocarbenium ion intermediates, which acceptors can attack at either the α or the β face. As insight into the nature of S_N1 reactions is still limited,[35] we focus our discussion on the influence of protecting groups on anomeric stereocontrol (see Section 3).

Other means are also often used to achieve high anomeric stereocontrol in glycoside syntheses.[4–6,36] The concept of in situ anomerization of halogenoses with axial halide to halogenoses with equatorial halide introduced by Lemieux et al.[37] in early studies proved to be a major breakthrough in the synthesis of *cis* glycosides: The activation of relatively stable α-glycosyl halides in the presence of quaternary ammonium halides leads to the establishment of an equilibrium with the more reactive β-glycosyl halides. The energy barrier to the nucleophilic substitution of β-glycosyl halides to give *cis* glycosides is lower than the corresponding transformation of α-glycosyl halides into *trans* glycosides; the net result is the preferred formation of *cis* glycosides. The influence of solvents on anomeric stereocontrol was also recognized very early on. In particular, the ether effect[4–6] and the nitrile effect[4a,38] play a major role in terms of the selectivity of the transformation.

Other parameters, such as temperature, pressure, concentration, and even the sequence of addition of the reactants, also have significant effects on the glycosidation selectivity.[39] Thus, optimization of the reaction conditions is frequently required for a particular glycosidation reaction for it to proceed with high stereoselectivity. Recent advances are highlighted in the appropriate context in this Review.

Efficient one-pot glycosylation protocols that enable the convenient assembly of oligosaccharides from appropriately protected building blocks in a minimum number of synthetic steps have received much attention in the past few years (see Section 4).[40] In combination with computational tools, this technique has been further developed into a programmable one-pot synthesis.[41] Solid-phase oligosaccharide synthesis is also an area of active investigation,[42] as it enables the rapid assembly of structures of interest with only a single purification step necessary (Section 5). A few research groups use this technique for the automated synthesis of oligosaccharides.[43] Enzymatic glycosidation is closely related to an intramolecular glycosyl transfer to the acceptor. Therefore, this concept has been investigated extensively in recent years (see Section 6).[44,45].

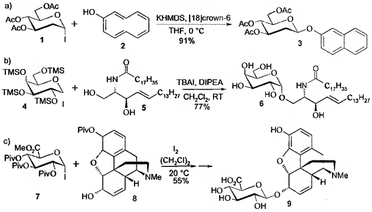
In spite of all these promising techniques in combination with other new techniques, such as product separation with the help of fluorous chemistry, there is still no general procedure for the stereoselective synthesis of glycosides and complex glycoconjugates. Therefore, the synthesis of these compounds often requires substantial know-how and systematic research.[4,46]

2. Glycosyl Donors and Activation Conditions

2.1. Glycosyl Iodides

Glycosyl halides were introduced as glycosylating agents by Koenigs and Knorr in 1901.[3] Glycosyl iodides were first prepared by the treatment of glycosyl bromides with sodium iodide in acetone more than half a century ago,[47] and new preparative procedures are still emerging.[48] Although glycosyl iodides have generally been considered too reactive to be of synthetic utility, several research groups have demonstrated that iodide donors display unique properties in glycosylation reactions and often offer advantages over glycosyl chlorides and bromides in terms of reaction time, efficiency, and the stereochemical outcome.[49] Various glycosides have been synthesized by using iodides as glycosyl donors, most notably by Gervay-Hague and co-workers, who also carried out mechanistic studies on the stereoselective formation of α,β-glycosyl iodides.[50] In general, iodide donors can be activated under basic conditions to give β-glycosides with high selectivity;[51] alternatively, in situ anomerization can be used for the selective synthesis of α-glycosides.[52] All glucosyl, galactosyl, and mannosyl iodides showed high reactivity towards strained oxacycloalkane acceptors in the presence of magnesium oxide. The corresponding glycosides were formed with high β selectivity.[53]

α-Glycosyl iodides have been shown to undergo in situ anomerization upon treatment with TBAI and Hünig base under standard conditions; the α-glycosides can then be prepared, even with sterically demanding acceptors, by nucleophilic substitution of the β-glycosyl iodide intermediates or through axial attack on the oxocarbenium ion intermediates. The utility of iodide donors was also demonstrated in the highly stereoselective synthesis of aryl 2-deoxy-β-glycosides, such as **3** (synthesized from **1** and **2**, Scheme 1 a).



Scheme 1. Glycoside syntheses with glycosyl iodides as donors.

Direct S_N2 displacement of the anomeric iodide circumvented the need to introduce at C2 temporary stereodirecting groups that would require subsequent removal.[54] The fully silylated galactosyl iodide **4** was also prepared and used to construct the biologically active α-glycolipid **6** in a highly selective fashion (Scheme 1 b).[55] To date, most glycosyl iodides used for glycoside synthesis have been protected with arming (activating) protecting groups (typically *O*-benzyl or electron-donating groups), although disarmed (deactivated) glucuronyl iodides, with electron-withdrawing groups on the pyranose ring, also proved to be efficient donors in β-glucuronylation reactions of a range of steroidal alcohols.[56] The reaction of the pivaloylated glucuronyl iodide **7** with 3-*O*-pivaloylmorphine (**8**) in the presence of iodine afforded the 1,2-*trans* glycoside stereospecifically in 55 % yield; subse-

quent deprotection gave morphine-6-glucuronide (**9**; Scheme 1c).[48d,138a] The synthesis of glucuronides of a drug candidate is often necessary to provide both an analytical standard for the quantification of metabolite levels in clinical samples and material for further pharmacological evaluation.

Recently, mannosyl iodides with participating groups at C2 were used to synthesize oligomannosides in the presence of AgOTf as an activator.[57] This process complements glycosidation with in situ anomerization. It demonstrates that iodide donors acylated at C2 are equally efficient and that the common base-induced side reaction of glycosyl iodides (i.e. elimination) can be suppressed.[52c] Glycosyl iodides have clearly become very useful glycosylating agents; in many cases, however, they can only be generated in situ owing to their high reactivity. Therefore, the stability of glycosyl iodides will need to be increased and other activation conditions developed before these compounds become widely used glycosyl donors.

2.2. Thioglycosides

Thioglycosides are frequently used as glycosyl donors in glycoside synthesis. Since the first report in 1909,[58] thioglycoside chemistry has been explored constantly. Numerous protocols have been reported for the preparation and activation of thioglycosides over the past century.[59] The advantage of thioglycosides lies in their great stability under a wide range of conditions for protecting-group manipulation. Anomeric thioether groups can thus act themselves as temporary protecting groups. Therefore, thioglycosides can serve not only as glycosyl donors, but also as glycosyl acceptors. This feature, combined with the tunable reactivity of thioglycosides, has often been exploited for the efficient synthesis of complex oligosaccharides.[60]

Thioglycosides are usually prepared by treating peracetylated sugars with the appropriate thiol in the presence of a Lewis acid, typically $BF_3{\cdot}OEt_2$.[61] An alternative synthetic route involving *S*-glycosyl isothiouronium intermediates[62] was reinvestigated for the preparation of alkyl thioglycosides: The intermediates were prepared from the corresponding glycosyl bromides and thiourea, and then converted into thioglycosides by S-alkylation in the presence of a mild base and an appropriate alkyl halide.[63] Thioglycosides have also been prepared by the treatment of glycosyl bromides with nucleophilic thiolates generated in situ through the zinc-mediated reduction of disulfides.[64]

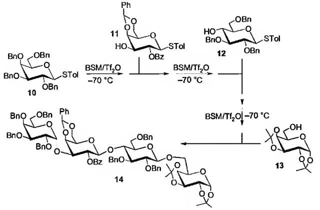
Thioglycosides can be activated by a wide range of promoters of variable reactivity. In all cases, at least a stoichiometric amount of the reagent is needed. A key contribution was made in 1990 by van Boom and co-workers,[65] who first reported the use of a stoichiometric amount or an excess of *N*-iodosuccinimide (NIS) in conjunction with a catalytic amount of triflic acid as a promoter to activate thioglycosides. The use of NIS/AgOTf was reported soon afterwards.[66] Since then, the efficacy of the iodonium system has been proven by numerous successful applications, and many variants have been developed (Table 1).[67–81] For example, the use of $HClO_4$ immobilized on silica as an alternative to HOTf for the activation of thioglycosides led to comparable results.[68] Mukaiyama and co-workers introduced the combined use of a stoichiometric amount of either NIS or NBS and a catalytic amount of $TrB(C_6F_5)_4$ as a promoter system.[69] Recently, another iodonium system, IPy_2BF_4/HOTf, proved to be effective for β-selective glycosidation reactions of perbenzylated armed thioglycosides. It was found to be compatible with one-pot sequential glycosylation reactions.[70] Furthermore, armed thioglycosides cospotted with sugar alcohols onto alumina TLC plates were converted into glycosides on exposure to I_2 vapor. The product was then purified by conventional elution with a solvent.[71] Interhalogen compounds (ICl or IBr) can be used in combination with AgOTf as a convenient and efficient promoter system for the activation of thioglycosides. High-yielding sialylation reactions with this system were described.[72] Thioglycosides have also been activated with other halonium systems; for example, a cheap bromonium system (stoichiometric NBS and catalytic $Bi(OTf)_3$) was used to activate various thioglycoside donors.[73] Commercially available 1-fluoropyridinium triflates successfully promoted the transformation of thioglycosides into *O*-glycosides.[74]

Table 1: Typical thiophilic promoters reported between 1998 and 2007.

Promoter	Ref.
NIS/$Sn(OTf)_2$ or $Cu(OTf)_2$	[67]
NIS/$HClO_4$–silica	[68]
NIS/$TrB(C_6F_5)_4$	[69]
IPy_2BF_4/HOTf	[70]
IX/AgOTf	[72]
NBS/$Bi(OTf)_3$	[73]
1-fluoropyridinium triflate	[74]
EtSNPhth/$TrB(C_6F_5)_4$	[75]
N-(phenylthio)-ε-caprolactam/Tf_2O	[76]
S-(4-methoxyphenyl)benzenethiosulfinate/Tf_2O	[77a]
BSP/Tf_2O	[77b, 78]
Ph_2SO/Tf_2O	[77c, 80]
BSM/Tf_2O	[77e]
Me_2S_2/Tf_2O	[81]

In the past decade, organosulfur compounds have become valuable promoters for thioglycoside activation: Early studies were devoted to sulfonium or sulfenyl triflates, such as DMTST, MeSOTf, and PhSOTf; more recently, sulfenamide activators in combination with Lewis acids such as EtSNPhth–$TrB(C_6F_5)_4$[75] and *N*-(phenylthio)-ε-caprolactam–Tf_2O[76] were proposed. Sulfinates in combination with Tf_2O have also received much attention as thioglycoside activators.[77]. For example, the system 1-benzenesulfinylpiperidine (BSP)/Tf_2O proved very useful for the synthesis of the Salmonella type E_1 core trisaccharide.[78] Ph_2SO/Tf_2O has been employed successfully for the synthesis of challenging sialic acid glycosides[79] and hyaluronic acid oligomers.[80] Recently, another powerful system, namely Me_2S_2/Tf_2O, was developed for the activation of thioglycosides.[81] An important feature of these sulfinyl systems is their capacity to preactivate thioglycosides at low temperatures.[82] Thus, one thioglycoside can be activated in the presence of another. Glycosylation reactions mediated by sulfinyl derivatives have been used to advantage in this way in the efficient synthesis of numerous complex

structures. For example, the tetrasaccharide **14** was constructed in less than 2 h from the thioglycoside building blocks **10–13** with benzenesulfinyl morpholine/Tf_2O as the promoter by use of the preactivation strategy (Scheme 2).[77e]



Scheme 2. Oligosaccharide synthesis with thioglycosides as donors.

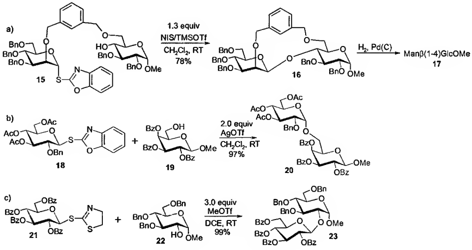
Other conditions for thioglycoside activation, such as the use of $AgPF_6$[83] and electrochemical oxidation,[84] have also been reported in the past few years.

In summary, numerous new methods for the preparation and activation of thioglycosides have been reported in the past decade. As thioglycosides are among the best Koenig–Knorr-type glycosyl donors, they will continue to play an important role in glycoside-bond formation in spite of the large quantities of highly reactive reagents required for their activation.

2.3. Glycosyl Thioimidates

Glycosyl thioimidates are glycosides that contain an $SCR^1{=}NR^2$ aglycone. Their preparation was first described more than 40 years ago,[85] and their use as glycosyl donors dates back to the late 1970s, when Woodward et al. used N-heterocyclic thioglycosides as glycosylating agents in the total synthesis of erythromycin (this synthesis was not published until 1981).[86] The glycosyl-donor properties of glycosyl thioimidates have since been investigated extensively.[87] Two classical routes to glycosyl thioimidates involve the Lewis acid promoted displacement of anomeric acetoxy groups with thiol aglycones or the displacement of anomeric halogen substituents with thiolate anions.[88] Both procedures are frequently used and high yielding.

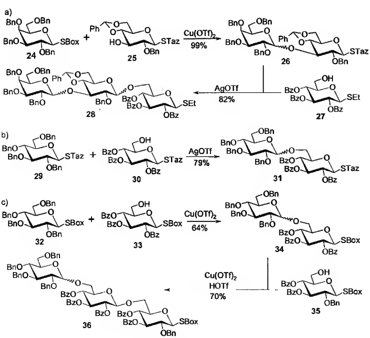
As thioimidate donors have the properties of both a thioglycoside and an imidate, conceptually different modes of activation are available. Thus, not only thiophilic reagents, such as NIS/TMSOTf, but conventional promoters for the activation of glycosyl thioimidates, such as $BF_3{\cdot}Et_2O$, have been used to activate thioimidate donors (Scheme 3). For example, a number of mannosyl thioimidates, including the *S*-benzoxazolyl (SBox) glycoside **15** and an *S*-benzothiazolyl glycoside, were prepared and activated effectively with NIS/TMSOTf for the synthesis of β-mannosides, such as **17**, through an intramolecular glycosylation (Scheme 3a).[89] *S*-Benzoxazolyl glycosides have also been activated with an excess of MeOTf or AgOTf ($\geq$ 2.0 equiv) in the synthesis of both 1,2-*trans*[90] and 1,2-*cis*[91] glycosides. Glycosylation of the galactosyl acceptor **19** with the SBox donor **18** in the presence of AgOTf afforded the disaccharide **20** with complete stereoselectivity in 97 % yield (Scheme 3b).[91] Interestingly, recent mechanistic studies indicated that both MeOTf and AgOTf activate this type of donor at the anomeric sulfur atom.[92] *S*-Thiazolinyl (STaz) glycosides, such as **21**, are also efficient glycosyl donors upon activation with AgOTf, $Cu(OTf)_2$, MeOTf, NIS/TfOH, or other promoter systems.[93] Their reaction with a variety of acceptors provided the corresponding glycosides in high yields (Scheme 3c). Furthermore, it was possible to activate one STaz leaving group chemoselectively in the presence of another by engaging one of the leaving groups in a stable palladium(II) complex.[94] In all of these reactions, it was absolutely necessary to use a stoichiometric amount of the promoter. The most common thiophilic reagent system, NIS with catalytic TfOH, did not even initiate the glycosylation with STaz donors. This reaction was only driven to completion by NIS in combination with stoichiometric TfOH.[93]



Scheme 3. Glycoside syntheses with glycosyl thioimidates as donors.

An advantage of thioimidate donors is their high stability. In general, they withstand the rather harsh reaction conditions often required for protecting-group manipulations, for example, through acetylation, benzylation, benzylidenation, or deacetylation. Therefore, glycosyl thioimidates containing different protecting groups can be prepared readily.[95] Unprotected hexofuranosyl thioimidates were also prepared

and found application in the synthesis of the corresponding glycosyl phosphates[96] and hexofuranosides.[97] Thioimidate donors can withstand reaction conditions associated with the activation of other glycosyl donors, such as thioglycosides, glycosyl bromides, and O-glycosyl trichloroacetimidates; thioimidates themselves can be activated selectively in the presence of thioglycosides and n-pentenyl glycosides (NPGs). These possibilities were used to develop rapid synthetic routes to oligosaccharides, as illustrated by the synthesis of trisaccharide **28** (Scheme 4a).[98] A conventional glycosylation



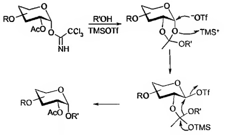
Scheme 4. Chemoselective activation of thioimidate donors.

strategy with armed and disarmed reaction partners has also been used with thioimidate donors,[98] whereby perbenzylated (armed) thioimidates, such as **29**, were activated chemoselectively in the presence of acylated (disarmed) thioimidates, such as **30** (Scheme 4b). Recently, an unusual reactivity pattern was observed for SBox glycosides: 3,4,6-Tri-O-acyl 2-O-benzyl SBox glycosides are significantly less reactive than even "disarmed" peracylated derivatives. This finding was then exploited with the "armed–disarmed" strategy to synthesize oligosaccharides of different linkage patterns, such as trisaccharide **36** (Scheme 4c).[99] Further investigation is required to determine the precise mechanistic details underlying these results; however, the absence of neighboring-group assistance probably accounts for the low reactivity of 3,4,6-tri-O-acyl 2-O-benzyl SBox glycosides in the presence of a relatively weak promoter.[100] Thioimidate donors have also been activated chemoselectively in the synthesis of sialosides[101] and galactofuranosides.[102]

Overall, glycosyl thioimidates are good glycosyl donors for which interesting applications have been found in oligosaccharide synthesis. As is the case for most glycosyl donors, a reduction in the required amount of the promoter should be the focus of future research.

2.4. 1,2-Orthoesters of Aldoses

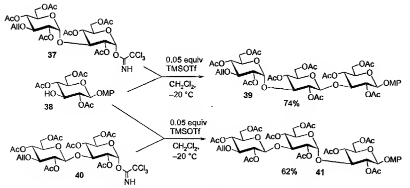
1,2-Orthoesters are often intermediates in glycosylation reactions. Their reversible formation is related to the Fischer–Helferich method. On acid-catalyzed anomeric activation of the glycosyl donor, the 2-O-acyl group undergoes dioxolenium formation. However, an acylative attack by the resonance-stabilized carbenium ion then takes place instead of an alkylative attack by the electrophilic anomeric carbon atom of the acceptor. The orthoester intermediates thus obtained can be transformed in situ into the desired glycosides by the addition of more of the catalyst, by extending the reaction time, or by other means.[103] This rearrangement usually leads to 1,2-*trans* glycosides through different possible pathways[104] and was used to develop new strategies for the regio- and stereoselective synthesis of oligosaccharides.[105] Nevertheless, additional studies, most notably by Wu and Kong, revealed that the rearrangement can also give 1,2-*cis*-linked products[106] in a transformation that does not conform with the classical concept of neighboring-group participation.[107] It is not yet clear how the unusual rearrangement proceeds, but a mechanism involving the formation of a β-glycosyl triflate was proposed (Scheme 5).[108] Also, remote stereochemical control was observed in the rearrangement of orthoesters; that is, the glycosidic bonds originally present in either the donor or the acceptor had a decisive influence on the configuration of the newly formed glycosidic linkage.[106b,109]



Scheme 5. Proposed rearrangement of orthoesters to form α-glycosides.

For example, the stereochemical outcome of (1→3) glucosylation reactions (**37**+**38** or **40**+**38**) could be controlled by varying the configuration of the glycosidic linkage present in the donor (Scheme 6). Orthoesters can not always be converted into the desired glycosides; sometimes they are isolated in high yield as by-products.[110]

In this section, we discuss the utility in glycoside synthesis of orthoesters activated as glycosyl donors according to the principles of the Koenigs–Knorr method. In the past, major improvements in this field resulted from the use of 1,2-



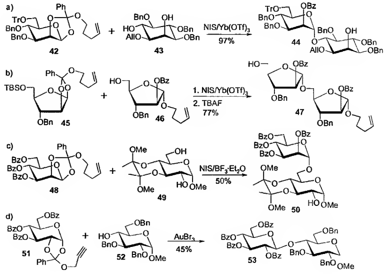
Scheme 6. Remote control of orthoester-mediated glycosylation reactions.

thioorthoesters[111] and cyanoethylidene analogues[112] as glycosylation agents that can be activated chemoselectively with an equimolar amount of a reagent specific for the thio or cyano group, respectively. However, these glycosyl donors did not gain wide application owing to the formation of by-products during the glycosidation and the highly toxic reagents used in their preparation. Therefore, for a long time, orthoesters have not been viewed as ideal glycosyl donors. Results of Fraser-Reid and co-workers with *n*-pentenylorthoesters (NPOEs) may change this view.[113] However, NPOE activation requires at least an equimolar amount of a pentenyl-group-activating reagent.

NPOEs were used originally as versatile synthetic intermediates which could undergo protecting-group manipulations under non-acidic conditions and be transformed afterwards into other glycosyl donors, in particular *n*-pentenyl glycosides (NPGs).[114] Soon these orthoesters derived from pentenyl alcohols were investigated thoroughly as glycosyl donors with different promoters and acceptors.[113] A major advantage of these donors is that activation with NIS leads through reaction with the iodonium ion to the liberation of the pentenyl moiety as iodomethyltetrahydrofuran, which can therefore not compete with the acceptor with respect to the formation of the glycosidic bond.

Various Lewis acid/NIS combinations have been examined as promoters for the activation of NPOE donors;[115] in general, $Yb(OTf)_3$/NIS gave the best results in terms of the yield and regioselectivity of glycosidation reactions and compatibility with protecting groups. For example, the *myo*-inositol diol acceptor **43** was mannosylated regiospecifically with the NPOE donor **42** in the presence of NIS/$Yb(OTf)_3$ to give the monomannosylated product **44** in almost quantitative yield (Scheme 7a). Both the selectivity and the yield dropped when other Lewis acids were used.[115c] Acid-labile protecting groups, such as cyclic acetals, were also preserved completely under NIS/$Yb(OTf)_3$ conditions.[115a] Moreover, this promoter system activated neither armed nor disarmed NPGs. NPGs could thus serve as acceptors towards NPOE donors, as illustrated by the synthesis of **47** (Scheme 7b).[116]

Another clear advantage of NPOE donors is that they can replace disarmed NPGs in glycoside synthesis and often perform better in terms of yield and selectivity.[117] Furthermore, disarmed NPGs are much less reactive and also prone to addition across the double bond, a problem never encountered with NPOE donors.[118] Even more interestingly, NPOE donors often exhibit much better regioselectivity than other glycosyl donors and sometimes even opposite regioselectivity in the glycosidation of a particular diol acceptor.[119] The cyclic-ketal-protected glucose diol **49** was glycosylated regioselectively with the mannosyl NPOE donor **48** to give only the 6-*O*-linked disacchar-



Scheme 7. Glycoside synthesis with NPOEs as glycosyl donors.

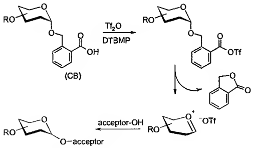
ide **50** in reasonable yield (Scheme 7c), whereas coupling with the corresponding phenylthioglycoside donor under the same conditions gave a mixture of 6-*O*- and 3-*O*-linked disaccharides, whereby the 6-*O*-linked isomer was produced in much lower yield.[119c] NPOEs glycosylate the equatorial hydroxy group of cyclic *syn* 1,3-diol acceptors specifically, whereas armed NPGs glycosylate predominantly the axial hydroxy group. Hence, the simultaneous treatment of the diols with both NPOE and armed-NPG donors led to only one trisaccharide of four possible products of double glycosidation.[119a] Rationalization of these regioselectivities led to the birth of the concept of reciprocal donor–acceptor selectivity (RDAS),[120] which is related to the concept of donor/acceptor matching introduced by Paulsen.[5]

The versatility of NPOE donors was further demonstrated recently in the efficient assembly of a pentadecamannan.[121] In this synthesis, NPOEs served not only as donors, but also as convenient intermediates in the generation of other glycosyl donors, such as trichloroacetimidates, thioglycosides, and NPGs. Propargyl 1,2-orthoesters, such as **51**, have also been reported recently as glycosyl donors (Scheme 7d).[122] The propargyl orthoester could be activated effectively with

$AuBr_3$ and gave different glycoside products in modest to good yields upon glycosidation with a series of acceptors.

2.5. Carboxybenzyl Glycosides

In 2001, Kim et al. reported a new type of glycosyl donor, 2-carboxybenzyl (CB) glycosides, which underwent glycosylation with high stereoselectivity in high yield (Scheme 8).



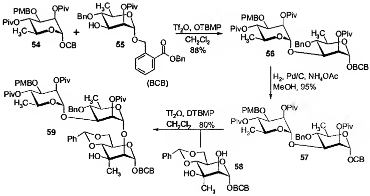
Scheme 8. Proposed mechanism for the activation of CB glycosides.

Previously, CB glycosides had been used to construct pentadienyloxy systems with very good glycosyl-donor properties.[123] In general, CB donors can be prepared readily by selective hydrogenolysis of their precursors, 2'-(benzyloxycarbonyl)benzyl (BCB) glycosides, even in the presence of other hydrogenation-sensitive protecting groups, such as benzyl and benzylidene groups. The BCB glycosides can in turn be synthesized from glycosyl bromides by the Koenigs–Knorr method, which increases the total amount of reagent required for glycoside synthesis. Alternatively, the anomeric O-alkylation method can be employed for the synthesis of BCB glycosides.

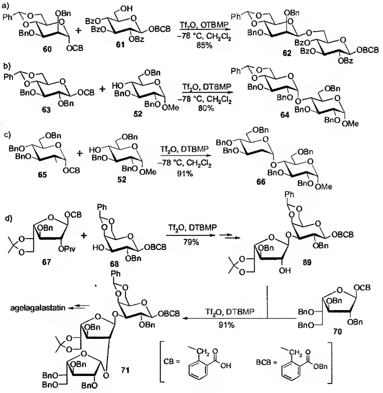
Conceptually, the glycosylation method described by Kim et al. is a variation of the method with *n*-pentenyl glycosyl donors. The driving force for the generation of oxocarbenium ions is the release of stable phthalide lactone through the action of Tf_2O/DTBMP, which must be used in at least equimolar amounts (Scheme 8).[124]

Unlike most glycosyl donors, CB glycosides can be activated in the presence of both acids and bases. In this way, many Lewis acid induced side reactions can be avoided.[4c] Another advantage is that the latent–active glycosylation strategy can be used successfully in combination with this method, because anomeric BCB groups are fairly stable under glycosidation reaction conditions but can be converted readily into CB leaving groups, as mentioned above. Thus, oligosaccharides could be assembled rapidly by employing latent BCB glycosides and active CB glycosides, as illustrated by the synthesis of trisaccharide **59** (Scheme 9).[125]

The utility of CB donors has also been demonstrated in the construction of numerous glycosidic bonds, including challenging β-mannoside and β-arabinofuranoside linkages. The β-mannoside **62** was produced exclusively and in high yield when the 4,6-*O*-benzylidene-protected CB mannoside **60** was used as the glycosyl donor with the acceptor **61** (Scheme 10a),[124] whereas glycosylation with the corresponding 4,6-*O*-benzylidene-protected CB glucoside **63** provided only α-linked glycosides, such as **64**, regardless of what kind of acceptor was used (Scheme 10b).[126] CB 2-deoxyglycosides have also been used as donors for the stereoselective synthesis of both α- and β-2-deoxyglycosides. In the synthesis of **66**, protecting groups on the donor played a pivotal role in the stereocontrol (Scheme 10c). The glycosylation properties of CB arabinofuranosides were also investigated, and high β selectivity was observed with glycosyl acceptors with 2-*O*-acyl protecting groups.[127] A stereospecific α-galactofuranosylation was used to form **71** in the total synthesis of agelagalastatin, an antineoplastic glycosphingolipid (Scheme 10d). CB glycosides were again employed as



Scheme 9. Oligosaccharide synthesis with CB glycosides as donors.



Scheme 10. Glycoside syntheses with CB glycosides as donors.

glycosylating agents in this synthesis.[128] 2′-(Allyloxycarbonyl)benzyl (ACB) glycosides have also been synthesized as latent glycosyl donors and used to construct complex oligosaccharides via the active CB glycosides.[129] In general, the anomeric configuration of CB glycosides did not affect the stereochemical outcome of their glycosidation.

2.6. Other Glycosyl Donors and Promoters

Since no single method is universally applicable and able to address all the issues associated with glycoside-bond formation, many other glycosyl donors, such as telluroglycosides,[130] glycosyl carbonates,[131] various heteroaryl glycosides,[15,21–25,132] various N-substituted glycosyl carbamates,[133] methyl 3,5-dinitrosalicylate (DISAL) glycosides,[134,135] glycosyl disulfides,[136] glycosyl sulfimides,[137] *N*-glycosyl amides,[138] glycosyl phthalates,[139] 2-allyloxyphenyl glycosides,[140] glycosyl 5-hexynoates,[141] and propargyl glycosides[142] have also been devised in the past decade. Furthermore, the development of new activation systems for existing donors propels carbohydrate chemistry forward. A variety of activation and promoter systems have been developed in the last decade (Table 2), some to simplify glycoside synthesis and others to improve glycosidation stereoselectivity. Of particular interest is the dehydrative glycosylation introduced by Gin and co-workers. This procedure starts, like the Fischer–Helferich method, directly from hemiacetals, which undergo irreversible in situ activation with a sulfonic acid anhydride and a sulfoxide in the presence of a base. It was applied successfully to various glycosidation reactions, including the synthesis of a complex saponin.[144a–d] However, the majority of the systems summarized in Table 2 still require a stoichiometric amount or even an excess of the promoter, and only a few applications have been reported.

Table 2: Promoters for other typical donors reported between 1998 and 2007.

Donor	Promoters (activation systems)	Ref.
glycosyl acetate	TMSI/$Ph_3P{=}O$	[143a]
	activated carbon fiber (ACF)	[143b]
1-hydroxy sugar	Ph_2SO/Tf_2O/2-Cl-Pyr	[144a]
	Me_2S/Tf_2O/TTBP	[144b]
	$n Bu_2SO/(PhSO_2)_2O$/TTBP	[144c]
	$CuCl_2$/dppf/$AgClO_4/CaSO_4$	[144e]
	CBr_4/PPh_3	[144f,g]
	Rh^{III}–triphos catalyst	[144h]
glycosyl fluoride	$LiClO_4$	[145a,b]
	TfOH/5-Å molecular sieves	[145c]
	$SnCl_2$ or $SnCl_4/AgB(C_6F_5)_4$	[145d,e]
	$HB(C_6F_5)_4$	[146]
	sulfated ZrO_2	[145f]
glycosyl bromide	I_2/K_2CO_3	[71b]
	$InCl_3$	[147a]
	tri(1-pyrrolidino)phosphine oxide	[147b]
glycosyl sulfoxide	Tf_2O/DTBMP/4-allyl-1,2-dimethoxybenzene	[148a]
	$[Cp_2ZrCl_2]/AgClO_4$	[148b]
	nafion-H or sulfated ZrO_2	[148c]
selenoglycoside	Br_2	[149a]
	electrochemical activation	[149b]

2.7. *O*-Glycosyl Imidates with Electron-Withdrawing Groups

Of the various synthetic strategies developed to date, glycoside syntheses based on *O*-glycosyl imidates, particularly trichloroacetimidates (often termed "Schmidt glycosidation"), are probably the most popular. *O*-Glycosyl trichloroacetimidates, introduced by Schmidt and Michel in 1980,[19] exhibit outstanding donor properties in terms of ease of formation, reactivity, and general applicability. High product yields and high anomeric stereocontrol are usually observed. The anomeric configuration of the product glycoside derives from the anomeric configuration of the *O*-glycosyl trichloroacetimidate (inversion or retention), anchimeric assistance, the influence of solvents, and/or thermodynamic or kinetic effects.[6] In 1984, Schmidt et al. reported another type of glycosyl imidate, namely, trifluoroacetimidates,[15,16] as glycosyl donors. Later, a series of N-substituted *O*-glycosyl trihaloacetimidates were also prepared from the corresponding glycosyl hemiacetals and N-substituted trihaloacetimidoyl chlorides.[21] Initial experiments revealed that glycosylation reactions with trifluoroacetimidates were generally less efficient than those with trichloroacetimidates in terms of product yield. Yu and Tao[150] and Iadonisi and co-workers[151] explored the application of *O*-glycosyl *N*-phenyltrifluoroacetimidates and reported particularly good reactivity for some specific glycosylation reactions. On the whole, trifluoroacetimidate donors are less reactive than the corresponding trichloroacetimidate donors, presumably as a result of the lower basicity of the nitrogen atom, the presence of a substituent on the nitrogen atom, and/or the smaller conformational changes caused by the trifluoromethyl group.[152] Recently, dichlorocyanoacetimidates were introduced as a new type of glycosyl donor with similar glycosylation properties to those of trichloroacetimidate donors.[17,18]

O-Glycosyl trichloroacetimidates can be prepared readily by a base-catalyzed addition of the anomeric hydroxy group to Cl_3CCN in the presence of either an inorganic or an organic base (generally NaH or DBU is used as the base; NaH is especially useful for the synthesis of donors with temporary Fmoc protecting groups, which would be cleaved by DBU). Recently, two research groups reported independently that polymer-supported DBU[153] and TBD[154] (1,5,7-triazabicyclo-[4.4.0]dec-5-ene) are efficient reagents for the preparation of trichloroacetimidates, which were obtained in excellent yield in pure form after simple filtration and evaporation. This method is particularly useful when the trichloroacetimidate donors formed are highly labile.[153] It was found in another investigation that polymer-bound DBU is most efficient under substoichiometric conditions and therefore the reagent of choice for the preparation of this important class of glycosyl donors.[155]

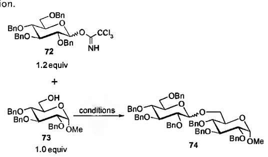
Trifluoroacetimidates in their N-unsubstituted form are more difficult to prepare than the trichloroacetimidates, as the corresponding reagent, trifluoroacetonitrile, is gaseous (b.p.: −64 °C) and toxic.[156] *N*-Phenyltrifluoroacetimidates

(PTFA)[21,157] have received much more attention and become the most common and most widely investigated trifluoroacetimidates. PTFA donors are usually prepared from anomeric hemiacetals in an irreversible reaction by treatment with *N*-phenyltrifluoroacetimidoyl chloride in the presence of a stoichiometric amount of a base. The use of K_2CO_3 as the base generally favors the formation of α-PTFA,[157] whereas the use of NaH[21] or DIPEA[158] yields mainly β products; however, more commonly, α/β mixtures are produced. Drawbacks of this method are the generation of an equimolar amount of a salt with the glycosyl donor and the irreversibility of glycosyl-donor generation; furthermore, structural assignments by NMR spectroscopy are difficult owing to the possible presence of invertomers and splitting of the signals of neighboring carbon atoms by fluorine.

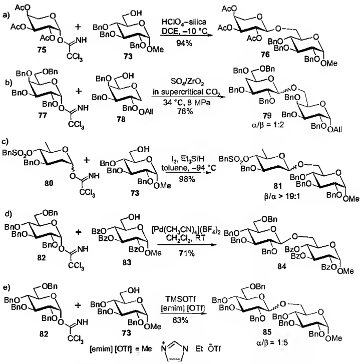
TMSOTf and $BF_3{\cdot}OEt_2$ are the most commonly used catalysts for glycosidation reactions of trihaloacetimidates. Several new catalysts for the activation of trichloroacetimidate donors have been reported in the past decade. Catalytic amounts of $Sm(OTf)_3$ activated armed *O*-glycosyl trichloroacetimidates under very mild conditions,[159] whereas disarmed trichloroacetimidates were activated effectively by $Yb(OTf)_3$.[160] These trivalent lanthanide triflates are generally stable salts that can be stored easily without particular precautions. AgOTf was also reinvestigated as a catalyst and found to be a mild and in some cases more efficient catalyst in TMSOTf-sensitive glycosylation reactions.[161] Beside the solvent, the nature of the counteranion in the catalyst has a major influence on the stereoselectivity of Schmidt glycosidation (see Table 3, entries 1–4 for the formation of **74**).[146] The reason behind this anion effect has not yet been elucidated.

Appropriately functionalized acyl sulfonamides were also employed as catalysts for glycosidation reactions with trichloroacetimidates.[162] More recently, silica-supported perchloric acid ($HClO_4$–SiO_2) was used as a convenient and efficient promoter in various glycosylation reactions with trichloroacetimidates as glycosyl donors (Scheme 11 a).[163] Also, the use of $HClO_4$–SiO_2 for "on-column" glycosylation and subsequent in situ separation provided a novel and robust

Table 3: Effects of catalysts on the stereoselectivity of Schmidt glycosidation.



Entry	Conditions	Yield [%]	α/β
1	$HClO_4$, Et_2O	99	91:9
2	$HB(C_6F_5)_4$, Et_2O	97	43:57
3	$HClO_4$, $PhCF_3$–*t*BuCN	95	54:46
4	$HB(C_6F_5)_4$, $PhCF_3$–*t*BuCN	97	10:90

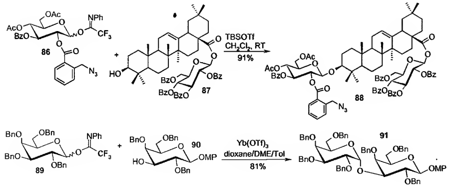


Scheme 11. New activation conditions for *O*-glycosyl trichloroacetimidates.

method for glycoside synthesis.[164] Reusable solid superacid in supercritical carbon dioxide (Scheme 11 b)[165] and amberlyst 15[166] were also employed successfully as activators. The direct and stereoselective synthesis of β-linked 2-deoxyoligosaccharides was achieved by the oxidative activation of glycosyl imidates. Glycosylation with 2-deoxyglycosyl trichloroacetimidates and HI generated in situ from I_2 and a catalytic amount of triethylsilane in toluene proceeded smoothly to provide the corresponding β-2-deoxyglycosides in excellent yield and with excellent selectivity (Scheme 11 c).[167] The air- and moisture-stable Lewis acid catalyst $[Pd(CH_3CN)_4](BF_4)_2$ was also used recently to access a variety of glycosides in good yields with excellent stereoselectivity. Notably, this catalyst directed β-glucosylation reactions without classical neighboring-group participation (Scheme 11 d).[168] Trichloroacetimidate donors were also activated by precise microwave heating in the absence of strong Lewis acids; the desired glycosides were formed in good yields.[134] A few studies were devoted to the use of ionic liquids as solvents (Scheme 11 e). The reactions proceeded at room temperature under mild conditions in these solvents, and the use of a Lewis acid catalyst could be avoided in some cases.[169]

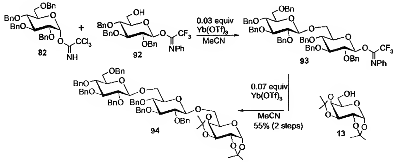
The "inverse procedure" developed by Schmidt and Toepfer in 1991[170] often provided the desired glycosidation products when glycosidation reactions otherwise failed to give glycosides or when orthoesters tended to form.[103] In the inverse procedure, it is thought that acceptor molecules aggregate around the catalyst, and that an intramolecular-type glycosylation takes place on the approach of the donor.[170]

Although most trichloroacetimidate activators, such as TMSOTf,[21] $BF_3{\cdot}Et_2O$,[21,171] TBSOTf,[172] $Yb(OTf)_3$,[173] and acid-washed molecular sieves,[174] can also be used to promote glycosidation reactions of PTFAs, the activation of PTFAs usually requires more forceful conditions. Two representative Lewis acid catalyzed PTFA glycosidation reactions are shown in Scheme 12. Some other activation systems, such as I_2/ Et_3SiH,[151] $Bi(OTf)_3$,[175] and $TMSB(C_6F_5)_4$,[176] have been used to promote PTFA glycosidation reactions. The different reactivity of PTFA and trichloroacetimidate donors was exploited to develop a one-pot multistep procedure featuring the selective activation of a trichloroacetimidate donor in the presence of a PTFA moiety.[177] The PTFA derivative **92** was partially protected to serve as a glycosyl acceptor in the first glycosidation step (Scheme 13).



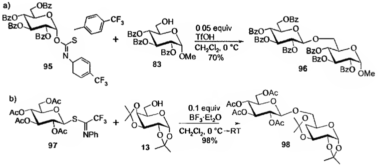
Scheme 12. Activation conditions for O-glycosyl trifluoroacetimidates.

N-Aryl-trifluoroacetimidate donors have in some cases shown advantages over trichloroacetimidates, for instance in the synthesis of β-mannosides[176] as a result of their lower propensity to undergo side reactions during glycosidation. In the course of trichloroacetimidate glycosidation, a certain amount of an N-glycoside by-product is occasionally produced through the glycosylation of trichloroacetamide liberated from the donor. This by-product can generally be removed readily by chromatography. The side reaction can be observed when the acceptor is of low nucleophilicity or sterically hindered but is diminished in PTFA glycosidation reactions owing to increased steric hindrance by the *N*-phenyl group. Therefore, PTFA donors exhibited excellent glycosylating properties in the synthesis of N-glycosides by the glycosylation of asparagine-containing peptides.[178] PTFA donors have also found application in many other oligosaccharide and glycoconjugate syntheses.[179]



Scheme 13. Selective activation of O-glycosyl trichloroacetimidates in the presence of PTFA donors.

Other types of *O*-glycosyl imidates, such as the thioformimidate **95** (Scheme 14a),[180] have also been investigated as glycosyl donors but have not gained much attention so far. In this context, the recent introduction of a new type of glycosyl thioimidate, *S*-glycosyl trifluoroacetimidates, enabled the catalytic glycosidation of *S*-glycosyl imidate donors. A catalytic amount of $BF_3{\cdot}Et_2O$ was sufficient to promote the glycosidation of S-glycosyl *N*-phenyltrifluoroacetimidate donors, such as **97** (Scheme 14b), which could be prepared readily from the corresponding glycosyl thiols. The glycosidation products were obtained in excellent yields.[181] Glycosyl thiols can be prepared readily from the corresponding reducing sugars by using the Lawesson reagent.[182] A highly stereoselective method for the synthesis of α-glycosyl thiols was also reported recently.[183] The availability and high configurational stability of both β- and α-glycosyl thiols should make them very useful for carbohydrate synthesis and particularly for the generation of glycosyl donors.



Scheme 14. Glycosidation reactions of other O-glycosyl imidates.

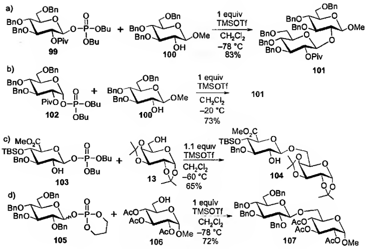
In many cases, the requirements for glycosidation reactions outlined in the Introduction are fulfilled by the trichloroacetimidate method:

- *O*-Glycosyl trichloroacetimidates are formed readily and generally stable at room temperature. However, under acid catalysis they are extremely good glycosyl donors.
- The release of nonbasic trichloroacetamide fulfills the criteria for acid catalysis: The acid is not consumed by the leaving group, and therefore generally only a catalytic amount of the (Lewis) acid is required.
- Trichloroacetamide is not acidic; therefore, the acidity of the reaction medium—determined by the catalyst

amount—is maintained throughout the course of the reaction.

- Glycosidation is basically a condensation reaction. In this procedure, water is bound to trichloroacetonitrile during trichloroacetamide formation. Hence, drying agents are not required.
- Trichloroacetamide can be removed from the reaction mixture and transformed back into trichloroacetonitrile. Thus, this method is cost-effective and environmentally friendly even on a large scale.
- Neither in the formation of the *O*-glycosyl trichloroacetimidates nor in the glycosidation reaction are equivalent or greater amounts of salts produced. Hence, expensive sterically hindered bases are not required.

Further studies on other *O*-glycosyl imidates with strongly electron withdrawing groups are required to fully evaluate their properties as glycosyl donors.



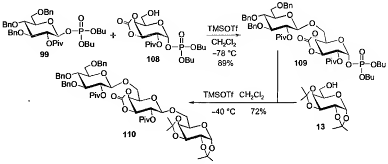
Scheme 15. Glycoside syntheses with glycosyl phosphates as donors.

2.8. Glycosyl Phosphates and Phosphites

The preparation of glycosyl phosphates, for example, by the treatment of *O*-glycosyl trichloroacetimidates with phosphorous acid, has attracted much attention since the early 1980s owing to their importance in biological processes.[184] A number of other approaches have since emerged.[185] Like trichloroacetimidate donors, both α- and β-glycosyl phosphates can be prepared readily, and they are stable enough to be stored for several months at 0 °C. The α isomers can be formed from the β isomers by acid-catalyzed anomerization. Protecting-group manipulations have been carried out directly on glycosyl phosphates, which makes the preparation of phosphate donors more flexible.[186]

To our knowledge, the first use of glycosyl phosphates as glycosyl donors was reported in the 1980s.[187] Schmidt and co-workers also investigated the glycosylation properties of glycosyl phosphates, which were found to be much less efficient donors than the corresponding *O*-glycosyl trichloroacetimidates.[188] Therefore, little attention was paid to the synthetic utility of glycosyl phosphates until more recently.[189] Seeberger and co-workers screened a series of Lewis acids as activators for glycosyl phosphate donors and came to the conclusion that only silyl triflate reagents, such as TMSOTf and TBSOTf, could ensure high-yielding glycosylation reactions. $BF_3{\cdot}Et_2O$ gave modest results, and the efficacy of protic acids, such as TfOH and TsOH, was very low.[190] Thus, the majority of glycosidation reactions with phosphate donors have been promoted by TMSOTf (Scheme 15). Unfortunately, at least a stoichiometric amount[191] of TMSOTf is often required to activate glycosyl phosphates, or even up to three equivalents.[182] The reason for the requirement of at least one equivalent of TMSOTf could be ascribed to the formation of a stoichiometric amount of a silyl phosphate, which is possibly the driving force for this type of glycosidation reaction.[190]

The more reactive β-glycosyl phosphate **99** could be activated at −78 °C, whereas the corresponding α isomer **102** usually required a higher temperature for activation (Scheme 15 a,b).[182] The reactivity difference between α- and β-glycosyl phosphates was used to develop an anomer-controlled orthogonal glycosylation strategy (Scheme 16),[190]



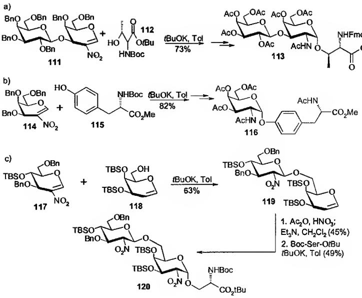
Scheme 16. Anomer-controlled glycosylation reactions with glycosyl phosphates.

which has not been reported for other glycosylation methods. Additionally, a regioselective glycosylation approach with phosphate donors was developed by using critical building blocks, such as **103**, with both donor and acceptor properties (Scheme 15 c). Such an approach minimizes the number of protecting-group manipulations required in oligosaccharide synthesis. Another interesting feature of phosphate donors is the formation of 1,2-*trans* glycosides at low temperatures, even with a nonparticipating group at the 2-position (Scheme 15 c,d).[191] Glycosidation probably proceeds via a close-ion-pair intermediate consisting of an oxocarbenium ion and a phosphate or triflate counter ion. The solvent effect of propionitrile was exploited to further enhance the β selectivity of the glycosylation with 2-azido-2-deoxyglycosyl phosphates.[192] Thus, in the construction of 1,2-*trans* and 1,2-*cis* glycosidic linkages, results with other glycosylation methods, such as the trichloroacetimidate method, could be transferred to the glycosylation with *O*-glycosyl phosphates.[193]

Glycosyl phosphites have been known for some time to function as glycosyl donors[194] but have received less attention than glycosyl phosphates. Some new activation systems and successful applications to the synthesis of 1,2-*cis* glycosides have been reported.[195]

2.9. Nitroglycals

Glycal chemistry was investigated extensively in the early 1990s and reviewed recently.[26,196] The focus of this section is on 2-nitroglycals, which have attracted much attention in the past decade.[34] Although 2-nitroglycals have been known for about 40 years,[197] their application in carbohydrate chemistry was limited until studies by Schmidt and co-workers in this field highlighted their potential. 2-Nitroglycals can be prepared readily from the corresponding glycals by the addition of acetyl nitrate generated in situ, followed by the base-promoted elimination of acetic acid.[198]



Scheme 17. Glycoside syntheses with 2-nitroglycals as donors.

The usefulness of 2-nitroglycals has been demonstrated in many cases. The most rewarding application is the base-catalyzed nitroglycal concatenation.[199] It is well known that the chemical synthesis of 2-acetamido-2-deoxy-α-D-galactopyranosides is difficult, as it necessitates a non-participating latent amino functionality at the 2-position of the glycosyl donor. As this α-glycosidic linkage is a common motif in numerous glycoproteins, particularly in mucins,[200] the development of an efficient synthetic route to such glycosides is of great significance. Recently, nitroglycal concatenation proved to be suitable for the synthesis of all core structures of the mucin family.[201] Moreover, this method, which consists of a mild acid-catalyzed glycosylation of a glycal, nitration of the enol ether moiety to generate a Michael-type acceptor, the highly stereoselective addition of a nucleophile (in particular, less-reactive alcohols), and subsequent reduction of the nitro group to an amino group, was extended readily to the synthesis of other complex glycosides (Scheme 17).

The Michael addition of serine and threonine derivatives to 2-nitrogalactal and derivatives, such as **111**, in the presence of KO*t*Bu gave the corresponding α-galactosides in high yields with high stereoselectivity. The products were subsequently transformed into useful building blocks for glycopeptide synthesis (Scheme 17a).[202] Similar coupling reactions promoted by weak bases, such as DBU and Et_3N, furnished mainly β-galactosides.[198] High stereoselectivity was also observed in the glycosylation of aromatic alcohols, including the tyrosine derivative **115** (Scheme 17b).[203] Other nucleophiles, such as lactates,[204] resonance-stabilized soft carbanions,[205] and dimethyl hydrogen phosphonate,[206] were added to 2-nitroglycals in a stereoselective fashion to give the corresponding glycosyl lactates, C-glycosides, and glycosyl phosphonates, respectively, in very good yields.[204–206]

This method has been applied to the synthesis of complex oligosaccharides. A range of complex glycals were synthesized by the glycosylation of simple glycals with trichloroacetimidate donors in the presence of the mild Lewis acid $Sn(OTf)_2$ and then exposed to the nitration conditions.[201,207] As expected, the resulting 2-nitroglycals underwent smooth Michael addition with different acceptors to give the corresponding glycosides in high yields with high stereoselectivity (Scheme 17).[207] As mentioned above, not only α but also β selectivity was possible in the glycosylation step. Scheme 17c shows a particularly interesting example: As a result of a conformational bias, the nitroglucal **117** underwent stereoselective attack by the galactal **118** to produce only the β-linked intermediate **119**, which was coupled with Boc-Ser-O*t*Bu, again through nitroglycal chemistry, to furnish the α-linked glycosyl amino acid **120**.[200] 2-Nitroglycals can be converted readily into 2-nitrothioglycosides, good glycosyl donors which provided mainly β-glycosides upon activation in the presence of different acceptors.[208]

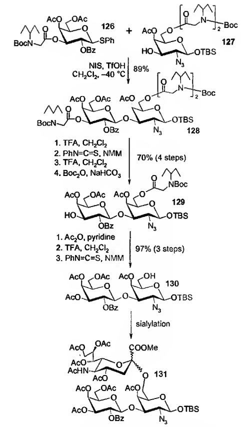
3. Protecting Groups

3.1. One-Pot Regioselective Protection and "Unichemo" Protection

Many of the steps required in glycoside synthesis involve the selective protection and deprotection of hydroxy groups (and sometimes amino groups). Therefore, protecting-group manipulation often takes up the most time in glycoside synthesis. This awkward situation may be improved at least in some instances by the impressive one-pot procedure developed initially by Hung and co-workers and later by Beau and co-workers for the regioselective protection of carbohydrates.[209] Sugar building blocks with different protection patterns were produced rapidly by this procedure, in which a single Lewis acid, TMSOTf or $Cu(OTf)_2$, was used to catalyze a sequence of reactions in a single reaction vessel. The desired building blocks were obtained by tuning the reaction con-

ditions. Thus, the TMS-protected glucoside **121** was converted efficiently into a series of acceptors, **122–125**, which contain chemically differentiable protecting groups (Scheme 18).[209a] This procedure was equally efficient on a large scale and could be adopted for other sugars as well. Glycoside synthesis can be speeded up greatly with this new protocol.[210]

Recently, another promising strategy originally developed by Miranda and Meldal, so-called unichemo protection (UCP),[211] was applied to carbohydrate synthesis. This strategy, which is illustrated in Scheme 19 for the synthesis of **131**, requires only one kind of protecting group and one deprotection procedure for oligosaccharide synthesis, because each hydroxy group is protected by a UCP group; the degree of polymerization of the amino acid derivatives differs.[212] Thus, the hydroxy groups could be liberated successively from the UCP groups from the lowest to the highest degree of polymerization by repeating the Edman degradation cycle. This protocol complements existing orthogonal-protection strategies and may be useful in oligosaccharide synthesis.



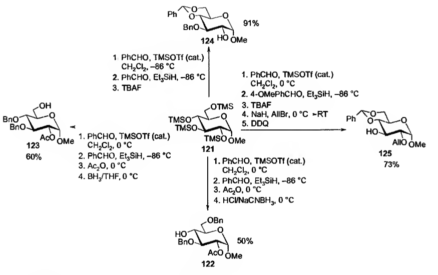
Scheme 19. Unichemo protection strategy for oligosaccharide synthesis.

3.2. *Protecting Groups That Do Not Impose Conformational Constraints*

In carbohydrate chemistry, the use of protecting groups goes far beyond the simple blocking of hydroxy groups. Protecting groups often play important roles in modulating the reactivity[213] of glycosyl donors and acceptors and directing the stereochemistry of glycosidation reactions. The focus of this section is on protecting groups that have a large influence on the stereocontrol of glycosidation reactions. These protecting groups can be classified tentatively into two main categories on the basis of their effects on the conformation of the sugar ring: protecting groups that do not impose conformational constraints (Section 3.2) and conformation-constraining protecting groups (Section 3.3).

3.2.1. *Neighboring Protecting Groups That Lead to 1,2-*trans *Glycosides*

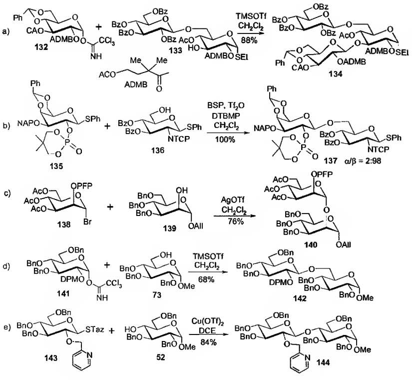
Neighboring-group participation by acyl groups is the most commonly used method for the construction of 1,2-*trans* glycosides. However, this method has drawbacks owing to the formation of orthoester by-products, which sometimes can not be converted into the 1,2-*trans* glycosides.[110]



Scheme 18. Examples of the one-pot regioselective protection of carbohydrates.

To avoid orthoester formation, several new 2-*O*-protecting groups have been developed for 1,2-*trans* glycosylation (Scheme 20). The bulky participating group 4-acetoxy-2,2-dimethylbutanoyl (ADMB) was used to protect the hydroxy group at the 2-position of glucose to prevent orthoester formation during glucosidation reactions through its sheer size and thereby enable the selective formation of β-glucosides (Scheme 20a).[214] The ADMB group could be removed under much milder conditions than those required for the commonly used bulky pivaloyl group (see below).

Dialkyl phosphates have been employed as stereodirecting protecting



Scheme 20. Neighboring protecting groups used in the synthesis of 1,2-*trans* glycosides.

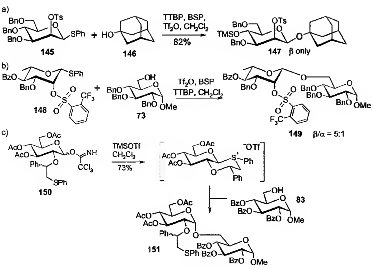
groups for the synthesis of 1,2-*trans* glycosides (Scheme 20b); neighboring-group participation of the phosphorous ester was proposed to account for the stereoselectivity observed.[215a] The phosphoryl protecting group was also used to mask the amino group of glucosaminyl trichloroacetimidates, which were converted upon activation mainly or exclusively into β products with a range of different acceptors.[215b] Stereospecific α mannosylation with mannosyl bromides equipped with a pentafluoropropionyl (PFP) group at the 2-*O*-position, such as **138**, has also been described (Scheme 20c).[216] The α selectivity is probably induced by neighboring-group participation and not by the electronic effect of the PFP group on the oxocarbenium intermediate, as the electronic effect should rather favor the formation of the β product (see Section 3.2.2).

When a new ether protecting group, diphenylmethyl (DPM), was introduced at the 2-*O*-position of glucose, the resulting glucosyl trichloroacetimidate **141** was converted exclusively into β-glucosides (Scheme 20d).[217] The steric bulk of the DPM group provides anchimeric assistance in the anomeric stereocontrol by shielding α-face attack by the nucleophile. The *S*-thiazolinyl glucoside **143** with a picolyl group at the 2-*O*-position was prepared and subjected to glycosidation conditions (Scheme 20e).[218] As expected, the picolyl group acts as an arming participating group, and β-glycosides were formed exclusively. With this method, both α- and β-anomeric pyridinium intermediates could be isolated, but only the former gave 1,2-*trans* glycosides. Other neighboring protecting groups, such as the 2-(allyloxy)phenylacetyl[219] and 2-(azidomethyl)benzoyl groups,[220] have been used to ensure 1,2-*trans* glycosylation reactions. A common feature of these groups and the ADMB group, and the driving force for their removal, is the release of a stable five-membered lactone or lactam upon deprotection.

With 2-aminosugars, neighboring-group participation by an acyl group often ends in the formation of relatively stable oxazoline by-products, which usually exhibit weak glycosyl-donor properties.[221] Therefore, many other N-protecting groups have been introduced to avoid the formation of these by-products, which impede glycoside-bond formation. A comprehensive review published recently on the synthesis of 2-aminoglycosides provides more information on these protecting groups.[222] The dimethylmaleoyl (DMM) group has attracted much attention as an N-protecting group owing to its excellent properties.[223] In contrast to the phthaloyl group, it can be removed readily with a base and subsequent treatment with an acid.

3.2.2. *Neighboring Protecting Groups That Promote the Formation of 1,2-*cis* Glycosides*

Ether protecting groups at the 2-*O*-position of a gluco- or galactopyranosyl donor favor generally the formation of 1,2-*cis* glycosides by virtue of the anomeric effect; hence, they are frequently used neighboring protecting groups in the synthesis of 1,2-*cis* glycosides. For corresponding 2-aminosugars, the azido functionality usually serves as an excellent masked form of the amino group. The influence of strongly electron withdrawing but nonparticipating sulfonyl protecting groups on the stereocontrol of glycosidation reactions has been reinvestigated in the last decade, most notably by Schmidt and coworkers and Crich et al.[224,225] On the whole, these protecting groups exhibit a good 1,2-*cis*-directing effect in glycosidation reactions with mannosyl and rhamnosyl donors. For example, β-mannosides could be prepared with a mannosyl trichloroacetimidate containing a benzylsulfonyl group at the 2-*O*-position or with mannosyl thioglycosides containing an aryl sulfonyl group at the 2-*O*-position as glycosyl donors (Scheme 21a).[225] These donors, upon activation, should favor the generation of a flattened twist-boat intermediate conformation (Figure 3) as a result of a strong dipole effect. This intermediate is then attacked preferentially from the β face to form a β-mannoside.[224] Although α-triflate intermediates were detected by NMR spectroscopy in similar glycosidation reactions, it is quite possible that the reactions did not proceed through an S_N2-type pathway as described, in view of the low to moderate β selectivity.[225] A more plausible mechanism involves the oxocarbenium ion intermediates mentioned above,[224] which are so reactive owing to the presence of strongly electron withdrawing groups that the selectivity is impaired. A similar situation was encountered in the activation of 2-fluoroglycosyl donors.[226] Moderate to good β selectivities were also observed in the glycosylation of a range of acceptors with rhamnosyl donors protected with sulfonyl groups at the 2-*O*-position, as exemplified in Scheme 21b by the reaction of **148**.[227] A trifluoroethylsul-



Scheme 21. Neighboring protecting groups used in the synthesis of 1,2-*cis* glycosides.

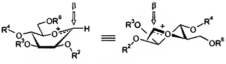


Figure 3. Flattened twist-boat intermediate.

fonyl group was also introduced at the 2-*O*-position of *O*-glucosyl trichloroacetimidates, but in this case the glycosidation selectivity was poor.[228]

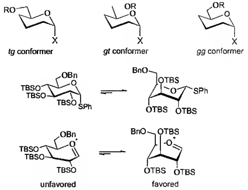
A new strategy for the stereoselective introduction of 1,2-*cis* glycosidic linkages was developed on the basis of *O*-glycosyl trichloroacetimidates with an (*S*)-1-phenyl-2-phenylthioethyl group at the 2-*O*-position, such as **150** (Scheme 21 c). These donors reacted through an unusual pathway: A quasistable anomeric sulfonium ion with a *trans*-decalin structure was formed through neighboring-group participation of the phenylthio group of the chiral auxiliary (Scheme 21 c). Thus, acceptors could only approach the sulfonium ion intermediate from the bottom face to give α-glycosides.[229] However, relatively harsh conditions were required to install and cleave this auxiliary. The early version of this auxiliary, the (*S*)-ethoxycarbonylbenzyl group, can also control the anomeric outcome of glycosylation reactions, probably via a similar *trans*-fused dioxenium ion intermediate; however, it was inferior to the (*S*)-1-phenyl-2-phenylthioethyl group in terms of 1,2-*cis* stereoselectivity.[230]

3.2.3. Non-neighboring Protecting Groups—Remote Stereocontrol

In the past few years, remote anchimeric assistance has also been exploited occasionally to control the configuration of the anomeric center during glycosylation reactions.[231] For example, a diethylthiocarbamoyl group was introduced at the 6-position of glucosyl fluorides and at the 4-position of galactosyl fluorides to shield the β face of the sugar rings and thus promote high α selectivity in glycosylation reactions.[231c] This long-range assistance is not discussed further herein, as in most cases it is not well established.

3.3. Conformation-Constraining Protecting Groups

As early as the beginning of the 1990s, Fraser-Reid et al. reported that cyclic acetals fused with *n*-pentenyl glycoside donors could deactivate glycosyl donors by imposing a torsionally disarming effect.[232] Since then, conformation-constraining protecting groups have been used frequently to increase or decrease the reactivity of donors and to enable orthogonal activation in the presence of other conventional armed or disarmed donors.[233] Recently, the cause of the disarming effect of 4,6-*O*-acetal groups on hexopyranosyl donors was scrutinized and attributed not only to torsional but also to electronic effects.[234] The acetal group, such as a benzylidene group, can lock the hydroxymethyl group in the *tg* conformation (Scheme 22), in which the C6–O6 bond acts as a dipole with the negative terminus directed away from the electron-deficient anomeric center in the transition state. This conformer is thus less reactive than the



Scheme 22. Conformation of the C6–O6 bond and super-armed donors.

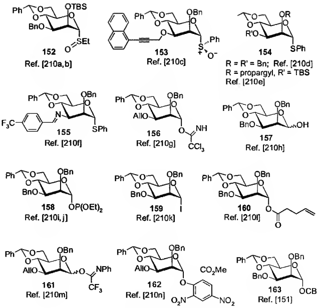
staggered *gt* and *gg* conformers, in which the dipole is much closer to being perpendicular to the developing positive charge. On the basis of the charge–dipole interaction theory, bulky silyl protecting groups, such as the TBS group, have since been introduced onto sugar rings to create so-called "super armed donors". The silyl groups force the ring to adopt a conformation rich in axial OR groups: a conformation which could have more-favorable charge–dipole interactions in the transition state (Scheme 22).[235] The resulting conformationally armed donors were more reactive than benzylated donors and could be activated selectively in the presence of other conventional armed or disarmed donors.

3.3.1. *Benzylidene Protecting Groups*

In recent years, conformationally constrained glycosyl donors have also gained attention as effective glycosylating agents for the construction of some glycosidic linkages that present a great synthetic challenge.[236] The rationale behind this technique is that, owing to the presence of conformation-constraining protecting groups, face-discriminating glcosyl cations are generated upon activation. These glycosyl cations can only be accessed from one side. In other words, anomeric stereoselectivity can be controlled by locking the donors into certain conformations.[237] Most of these donors contain cyclic bifunctional protecting groups,[238] which restrict the flexibility of the sugar ring to favor a certain conformation of the intermediary oxocarbenium ion. For example, various 4,6-*O*-benzylidenated mannosyl donors were reported by Crich and co-workers as highly β-selective mannosylating agents (Scheme 23).[176,239] Crich and co-workers first employed the sulfoxide donor **152**[239a] and the thioglycoside donor **154**[239d] to construct β-mannosidic linkages directly. They proposed that the reactions proceeded via an α-glycosyl triflate intermediate (or possibly its contact ion pair),[240] which led to preferred displacement from the β face, as the 4,6-*O*-benzylidene moiety opposed rehybridization of the anomeric carbon atom. Subsequently, Weingart and Schmidt also observed high β selectivity with the corresponding trichloroacetimidate donor **156** and a catalytic amount of TMSOTf as the promoter.[239g] The intermediacy of a conformationally constrained twist-boat structure was proposed to account for the remarkable β selectivity; that is, the anomeric stereocontrol is caused by a conformational effect enforced by the benzylidene group and not by reactions with α-glycosyl triflate intermediates. This mechanistic proposal reconciles all results found to date with different 4,6-*O*-benzylidene-protected mannopyranosyl donors.[239g] Furthermore, an investigation into the origin of the high β selectivity of glycosidation reactions of benzylidenated 2-deoxy-2-iodoglucosyl donors pointed to a similar twist-boat intermediate and ruled out the glycosyl triflate pathway.[241]

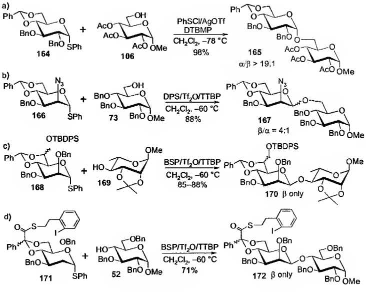
4,6-*O*-Benzylidene-directed β mannosylation reactions were carried out with the phosphite donor **158**,[239i] the iodide donor **159**[239k] and the PTFA donor **161**[176]in the presence of nontriflate promoters without the formation of mannosyl triflate intermediates. These results further support the argument that the conformational preferences of reaction intermediates could direct the stereochemistry of glycosidation reactions. Nevertheless, as in other glycosylation reactions, the leaving group,[239] activation conditions,[239m] protecting groups, substituent at the 2-/3-position,[242] and glycosyl acceptor[239g] can all affect the stereoselectivity of β mannosylation reactions to different extents.

Benzylidene protecting groups were also employed to restrict the conformation of other types of glycosyl donors, which upon activation reacted with acceptors to give the corresponding glycosides in a highly selective fashion. For example,



Scheme 23. 4,6-*O*-Benzylidenated mannopyranosyl donors.

glycosidation of the 4,6-*O*-benzylidenated thioglucoside donor **164** with a range of acceptors gave α-glucosides with excellent selectivities (Scheme 24a).[243] These results seem to stand in sharp contrast to the β-selective mannosylation reactions described above. However, in these reactions and most glycosidation reactions, a continuum of intermediates with different stabilities and lifetimes are likely to exist, each of which has its own reactivity and selectivity. The equilibrium proportions are highly dependent on a number of factors, including the leaving group, promoter, and reaction conditions. However, the conformational effect enforced by the benzylidene group contributes undoubtedly as a major factor towards the observed stereoselectivities. α-Glucosaminides were also synthesized stereospecifically by utilizing the



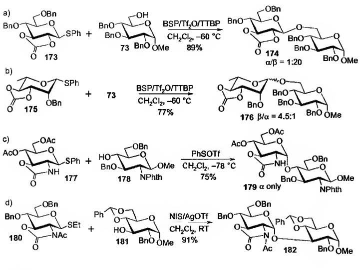
Scheme 24. Glycosidation reactions of benzylidene-constrained donors.

restraining effect of a 4,6-*O*-benzylidene group on *n*-pentenyl glycoside donors.[244] The same strategy was applied to the synthesis of β-mannosaminides. Surprisingly, in this case the degree of stereoselectivity seemed to be governed mainly by the configuration of the acceptor (Scheme 24 b).[245]

The direct stereocontrolled synthesis of the D- and L-*glycero*-β-D-*manno*-heptopyranosides **170** was carried out successfully by making use of the benzylidene acetal effect (Scheme 24 c).[246] The configuration at C6 had little effect on the stereochemical outcome. Crich and co-workers also developed a procedure for the synthesis of β-D-rhamnosides. In this synthesis, a stereoselective β mannosylation directed by modified benzylidene or alkylidene groups (Scheme 24 d) was followed by a tin-mediated radical fragmentation.[247] Again, the conformationally disarming acetal group promoted strong β selectivity.[247b]

3.3.2. *Carbonate and Oxazolidinone Protecting Groups*

The stereodirecting effect of the benzylidene group inspired the reinvestigation of carbonate and oxazolidinone groups with regard to their influence on the anomeric configuration. Thioglucosides protected with a 2,3-cyclic carbonate turned out to be good α-glucosylating agents with solvent assistance,[248] as no neighboring-group participation by the fused carbonate ring is possible during glycosidation reactions. Like the 4,6-*O*-benzylidene group, the carbonate group deactivates these thioglucosides both electronically and conformationally; hence, these donors can be used as acceptors in chemoselective glycosylation reactions with other 2-*O*-alkylated or 2-*O*-acylated thioglycosides. However, in the absence of the solvent effect, these donors showed good β selectivity and enabled the synthesis of β-glucosides, such as **174**, without recourse to neighboring-group participation (Scheme 25 a).[249] Apparently, conformational factors play a significant role in the anomeric stereoselectivity of these glycosylation reactions; however, the importance of glycosyl triflate intermediates has not yet been verified.



Scheme 25. Glycosidation reactions of constrained donors containing carbonate and oxazolidinone groups.

The 2,3-carbonate group was deemed torsionally arming in the rhamno- and mannopyranose series, because the half-chair conformation of the sugar ring imposed by the *cis*-fused carbonate ring lowers the activation barrier to oxacarbenium ion formation.[250] However, in both series, α-glycosides were formed predominantly as a result of a conformational effect. When thiorhamnoside 3,4-carbonates, such as **175**, were used as donors, β-glycosides became the major product (Scheme 25 b). This reactivity was attributed to the electron-withdrawing nature of the carbonate group and its inability to take part in neighboring-group participation.[250]

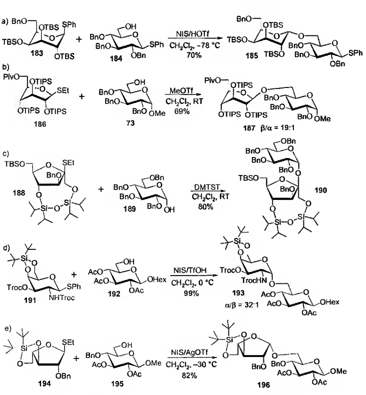
The oxazolidinone group has also attracted much attention as a stereodirecting group in glycosylation reactions. Kerns and co-workers first demonstrated that 2,3-oxazolidinone-protected thioglycosides, such as **177**, were highly efficient substrates for the synthesis of α-linked 2-amino-2-deoxyglucopyranosides (Scheme 25 c).[251a] The fused carbamate ring proved to be a nonparticipating group and favored the formation of α products. However, a limitation of this method was the propensity for N-glycosylation; that is, sometimes the oxazolidinone nitrogen atom was glycosylated.[251b] Therefore, the corresponding N-acetylated thioglycosides, such as **180**, were evaluated as glycosylating agents with different activation systems. The stereochemical outcome of glycoside-bond formation was found to depend on the relative reactivity and steric demand of the acceptors under BSP/Tf_2O/TTBP conditions.[252a] The mechanistic details of the reaction have not been reported; however, glycosyl triflate intermediates and steric hindrance by the *N*-acetyl group were invoked to explain the observed selectivities. Interestingly, the corresponding N-acetylated bicyclic donors showed complete β selectivity when NIS/AgOTf was used as the activation system, regardless of the reactivity of the acceptor. Moreover, the β products could be anomerized in situ to α products by using a larger quantity of AgOTf (0.4 equiv) to provide a convenient route to α-glucosaminides (Scheme 25 d).[252b] More recently, the glycosylation properties of *N*-benzyl-2,3-oxazolidinone-fused thioglycosides were investigated; on the whole, high α selectivities were observed.[253]

4,5-Oxazolidinone-protected thiosialosides proved to be excellent α-sialylating agents of various acceptors, even in the absence of the acetonitrile effect and neighboring-group participation by an auxiliary.[254] These results again indicate that conformationally constraining protecting groups play a significant role in the stereochemical control of glycosidation.

3.3.3. *Silyl Protecting Groups*

Silyl groups are very popular protecting groups in carbohydrate chemistry; however, until recently, their ability to control the conformation of sugar rings has not been utilized systematically to design efficient and stereoselective syntheses of glycosides. In the 1990s, several research groups observed that the naturally stable sugar-ring conformation, typically $^4C_1$ or $^1C_4$, could be flipped by the introduction of bulky silyl groups onto vicinal hydroxy groups of the sugar to give

the opposite or an unusual conformer,[255] in which most substituents have an axial orientation as a result of steric repulsion between the bulky protecting groups. Furthermore, glycosidation of the silyl-protected donors often gave preferentially or exclusively one stereoisomer, even without neighboring-group participation.[256] For example, upon activation, the tri-*O*-TBS-protected "super-armed" thiogalactoside **183** reacted with different acceptors stereospecifically to furnish α products (Scheme 26 a).[235a] A highly β-selective glucosylation method was also developed recently with tri-*O*-TIPS-protected thioglucoside donors, such as **186**, whose rings were constrained in a twist-boat conformation (Scheme 26 b).[256c] This method found application in the synthesis of 2-*O*-glycosylated glucosides.[256d]



Scheme 26. Glycosidation reactions of constrained donors containing silyl groups.

Cyclic bifunctional silyl groups have also often been used as protecting groups. They exert their influence on the stereochemistry of glycosidation by rigidifying the conformation of the sugar ring. An interesting example is the stereospecific synthesis of sucrose with 1,1,3,3-tetraisopropyldisiloxane-protected thiofructofuranosides, such as **188**, as glycosyl donors (Scheme 26 c).[257a] The α face of the donor is blocked by the internal silyl acetal bridge to ensure complete β glycosylation. This procedure was applied subsequently to the synthesis of β-linked oligofructofuranosides.[257b] Another cyclic silyl protecting group, the di-*tert*-butylsilylene (DTBS) group, was introduced into carbohydrate chemistry by Nishimura and co-workers in 2001.[258] It has attracted much attention owing to its strong stereodirecting effect on glycosidation reactions. For example, the glycosidation of 4,6-*O*-DTBS-protected galactosyl donors, such as **191**, with various acceptors gave the corresponding α-galactosides with very high selectivities.[259] High α selectivities were even observed in the presence of neighboring participating groups (Scheme 26 d),[259b] which indicates that the DTBS group has a very strong effect.

The DTBS group was also used to control the anomeric configuration in the formation of L-arabinofuranosides; the donors were locked in the E_3 conformation by the introduction of a fused DTBS ring at 3-O and 5-O.[260a] The conformationally constrained donor **194** gave excellent β selectivity in a range of glycosylation reactions with glycosyl acceptors containing primary and secondary hydroxy groups (Scheme 26 e). This method was employed successfully to synthesize an arabinogalactan fragment derived from the plant cell wall.[260a] It also has great utility and potential for β-D-arabinofuranoside synthesis, as evidenced in the synthesis of the arabinan domains of mycobacterial arabinogalactan and lipoarabinomannan. In these syntheses, two β-D-arabinofuranosidic linkages were constructed at the same time by the use of a similar DTBS-constrained donor.[236d,261] β-Selective D-arabinofuranosylation with 3,5-*O*-TIPDS-protected thioarabinofuranosides as glycosyl donors has also been reported.[262]

4. One-Pot Glycosylation

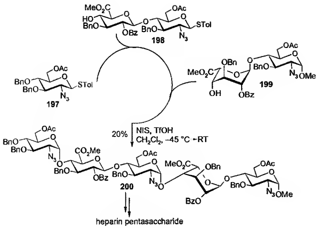
One-pot glycosylation is a highly useful approach in oligosaccharide synthesis. It relies on the reactivity disparity of glycosylating agents. Three major strategies have been employed most frequently in one-pot synthesis:

- Chemoselective glycosylation exploits the different reactivities of glycosyl donors and acceptors on the basis of the armed–disarmed concept;
- orthogonal glycosylation is based on the selective activation of a leaving group;
- in preactivation-based glycosylation, the glycosyl donor is activated separately, before the addition of the acceptor, which contains a leaving group for the next glycosylation step.

This topic was reviewed recently.[40] The above strategies and progress in the field are discussed in that article and in other relevant reviews.[60,263] Generally, two or three different glycosidic linkages are constructed in a one-pot process based on these procedures. A highly efficient chemoselective one-pot synthesis of heparin and heparan sulfate oligosaccharides through the use of thioglycosides with well-defined reactivity as building blocks (Scheme 27, synthesis of the pentasaccharide **200**),[264] shows the attractiveness of this approach.

5. Solid-Phase Oligosaccharide Synthesis

Initial attempts at solid-phase oligosaccharide synthesis (SPOS) in the early 1970s[265] met with little success owing to the limited range of glycosylation methods available. Solid-phase synthesis was not explored intensively until much later,

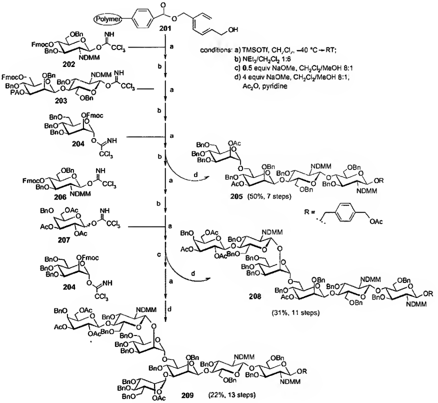


Scheme 27. One-pot synthesis of a heparin pentasaccharide.

when several new methods emerged. A few research groups have had substantial success in this field.[266] This section highlights significant results reported in the past decade, but does not cover all aspects of SPOS.

In the early 1990s, Danishefsky and co-workers successfully explored the application of the glycal assembly method to SPOS,[267] whereby the sugar chain was elongated from the nonreducing end. The first glycal unit was linked to a divinylbenzene–polystyrene copolymer through a disilane linkage that could be cleaved readily after the completion of the synthesis by treatment with fluoride. This protocol is self-corrective, as unused donors in a coupling step do not reemerge in the next cycle; it is particularly powerful for the synthesis of sugars branched at C2. However, 2-aminoglycosidic linkages of great biological importance could not be constructed directly by this procedure without further manipulation at the anomeric center.[268] Furthermore, this sort of donor-bound strategy could not simply be extended to other donors, as most side reactions during glycosylation reactions involve the donor. This strategy can easily result in the termination of chain elongation[269] and is therefore seldom used in SPOS.[270] It has been employed occasionally together with the acceptor-bound strategy to synthesize relatively short oligosaccharides by the so-called bidirectional approach.[271] In contrast, acceptor-bound strategies have been investigated in some detail, most notably by Schmidt and co-workers and Seeberger and co-workers, owing to their clear advantages: The donor can be used in excess to maximize glycosidation yields, and any donor-derived by-products can be washed away after each coupling step.

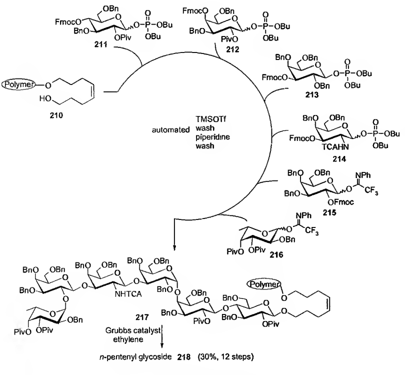
Most common glycosyl donors have been investigated as glycosylating agents in the acceptor-bound approach for SPOS, such as glycosyl sulfoxides,[272] *O*-glycosyl trichloroacetimidates,[273] thioglycosides,[274] *n*-pentenyl glycosides,[275] and glycosyl phosphates.[276] Enormous progress has been made with trichloroacetimidate-based SPOS. One important advance was the successful preparation of *O*-glycosyl trichloroacetimidates with an *O*-Fmoc protecting group and the demonstration of their suitability for oligosaccharide synthesis on a solid support.[277] A series of *N*-glycan oligosaccharides were synthesized on the Merrifield resin with the hydroxymethylbenzyl benzoate spacer–linker system **201**.[278a] Stereospecific glycosylation reactions with three types of trichloroacetimidate donors enabled chain extension (with **202**, **204**, and **206**), branching (with **203**), and chain termination (with **207**; Scheme 28).[278] For chain-branching donors, such as **203**, Fmoc and phenoxyacetyl (PA) were used as temporary protecting groups, with Ac, Bz, Bn, and *N*-DMM as permanent protecting groups. The crude saccharides released from the resin were of high purity after all glycosylation and protecting-group-manipulation steps. The simplicity and efficiency of the whole synthesis provided a basis for the development of a general approach to the synthesis of oligosaccharides with different glycosidic linkages. For example, a similar strategy was applied to the synthesis of a branched lacto-*N*-neohexasaccharide that occurs in human milk.[279] The release of the product from the resin as a benzylic glycoside made further deprotection easy. The key building block in the synthesis, *O*-lactosyl trichloroacetimidate, was protected orthogonally with Fmoc and Lev groups to enable selective glycosylation at both positions. Moreover, all trichloroacetimidate glycosidation reactions on the solid support were highly stereoselective and



Scheme 28. Solid-phase synthesis of N-glycans.

high-yielding, and the hexasaccharide was furnished in excellent overall yield. The great utility of Fmoc-protected O-glycosyl trichloroacetimidates has also been demonstrated in the synthesis of other oligosaccharides, such as oligomannosides,[280] lactosamine- and lactose-containing oligosaccharides,[280b] and glycosylphosphatidylinositol precursors.[281] Some other techniques have also been developed for SPOS in combination with the Schmidt glycosylation protocol, including on-resin real-time reaction monitoring[282] and novel capping reagents.[283]

Another major breakthrough in carbohydrate chemistry was the appearance of the first automated oligosaccharide synthesizer.[284] In 2001, Seeberger and co-workers carried out the automated synthesis of oligosaccharides by using a solid-phase synthesizer with O-glycosyl trichloroacetimidates and phosphates as glycosylating agents.[284] This synthesizer could assemble oligosaccharides as large as dodecasaccharides about 20 times faster than conventional methods through a simple coupling–deprotection cycle. A number of structures of biological relevance have been prepared automatically in this way.[285] For example, the tumor-associated carbohydrate antigen globo H was assembled successfully as the protected form **217** in six consecutive glycosylation reactions (Scheme 29).[285d] The hexasaccharide **218** was cleaved as its n-pentenyl glycoside from the octenediol-functionalized Merrifield resin with the Grubbs catalyst. A tetrasaccharide fragment of malarial toxin was also synthesized rapidly with this synthesizer by using the trichloroacetimidate method.[285h] However, the time-consuming preparation of the required glycosyl donors has not yet been improved by this technical advance.



Scheme 29. Automated synthesis of the tumor-associated antigen globo H.

SPOS has also benefited from other technical improvements, such as the above-mentioned on-resin analytical methods,[286] the capture–release purification technique,[287] and many new linker systems.[288] Recently, the hydrophobically assisted switching phase (HASP) concept was introduced into oligosaccharide synthesis. This procedure combines the great efficacy of solution-phase reactions with the high efficiency of solid-phase purification.[289] A discussion of all these aspects is beyond the scope of this Review. Polymer-supported oligosaccharide synthesis, particularly in view of the homogeneity of the reaction mixtures in solution, is also not discussed herein.

6. Intramolecular Glycoside-Bond Formation

The large number of possible oligosaccharide isomers results not only from the number of sugar monomers, but also from their varying regioisomeric (for example, connection to the 2-OH, 3-OH, 4-OH, and/or 6-OH group of hexopyranoses) and stereoisomeric linkages (α and β configuration). Although the advantage of intramolecular reactions for regio- and stereoselectivity is well known, for instance in asymmetric induction, and enzymatic glycosidation is closely related to an intramolecular glycosyl transfer from the donor to the acceptor, intramolecular glycosylation reactions have only been reported in about the last decade.[44,45] The published methods can be divided into three types of spacer-mediated reaction to form a linkage between the acceptor and the donor (Figure 4a–c).[44,45,290]

6.1. Leaving-Group-Mediated Reaction between the Donor and the Acceptor

In this method, the glycosyl acceptor is attached through a spacer Y to the leaving group of the glycosyl donor (Figure 4a). When the leaving group is released, the accepting oxygen atom is transferred with the attached glycosyl group to the anomeric carbon atom with heterolytic cleavage of the O–Y bond. The fragment $^{-}$L–Y^{+} that is formally released has to be designed to ensure stabilization, particularly of the Y^{+} moiety; otherwise, the heterolytic cleavage of the O–Y bond will not take place.

The connection of the anomeric hydroxy group of the glycosyl donor to the accepting oxygen atom of the acceptor through a carbonyl tether was used for a decarboxylative glycosylation.[291] Treatment of the resulting mixed carbonate esters with an acid led to the formation of glycosides with the loss of carbon dioxide.[292] Generally, yields are high in this reaction; however, competition experiments showed that the reactions are at least partially or even completely intermolecular processes.[293] Similarly, a tether between donor and acceptor was constructed by using 2-fluoro-3,5-dinitrobenzoic acid; in this case, glycoside-bond formation (though only in modest yield) simply requires heating in a polar solvent.[294]

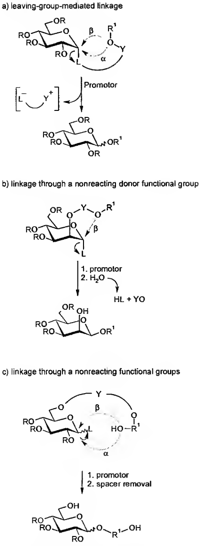
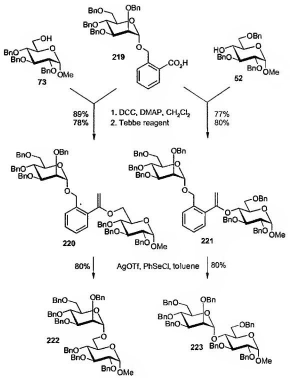
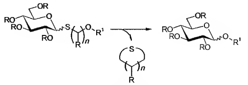


Figure 4. Different classes of spacer for linking glycosyl donors and acceptors.



Scheme 30. Pentadienyl-type linkage between a mannosyl donor and a glucose acceptor.



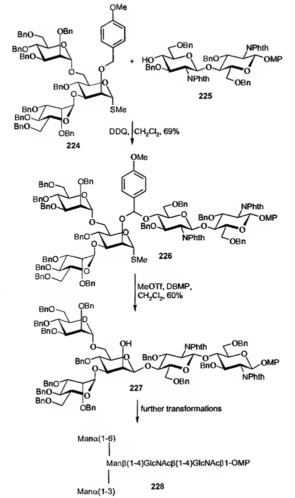
Scheme 31. Thioglycoside linkage between the glycosyl donor and acceptor.

Alternatively, 1-*O*-glycosyl esters with the acceptor bound to the β or γ position of the ester moiety enabled lactone formation by the released $^{-}L–Y^{+}$ fragment.[295] Pentadienyl-type activation was also investigated with different systems (Scheme 30).[123] Intermediates **220** and **221** provided excellent glycosylation results; however, these reactions were again found to be mainly intermolecular. Similar observations were made with thioglycoside donors that could undergo glycoside-bond formation through an intramolecular 1,3-, 1,4-, 1,5-, or 1,9-shift (with $n = 1, 2, 3, 7$ in Scheme 31).[296] Thus, this straightforward concept for intramolecular glycosylation has not yet furnished the desired results.

6.2. Linkage of the Glycosyl Donor and Acceptor through a Functional Group on the Donor

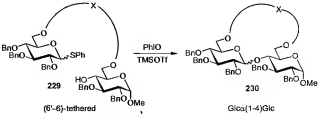
In this design (Figure 4b), the accepting oxygen atom is attached through a spacer Y to a non-anomeric functional group on the donor (generally the 2-O atom of the donor). When the leaving group is released, linkage of the donor to the accepting oxygen atom of the acceptor leads to cleavage of the Y–O moiety, whereby Y has a positive charge and requires stabilization. Final aqueous workup yields the product together with HL and YO. Several spacers Y have been studied, and different terms have been proposed for these reactions: "intramolecular or internal aglycone delivery" (IAD), "temporary silicon connection method", "silicon-tethered intramolecular glycosylation", "functional-substituent-based intramolecular glycosylation".[44,297]

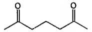
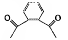
The IAD concept was originally developed for the synthesis of β-mannosides and later extended to other systems. The most common Y groups are isopropylidene, propylidene, ethylidene, 4-methoxybenzylidene, naphthylmethylidene, and dimethylsilylene. Particularly the use of the two arylidene groups led to excellent results (Scheme 32, transformation of **226** into **227**).[298] The high yields and β selectivities in mannopyranoside synthesis offer strong support for the intramolecularity of these reactions. However, to the best of our knowledge, this claim has never been confirmed by competition experiments.



Scheme 32. Linkage of the glycosyl donor and acceptor through a 4-methoxybenzylidene group.

Table 4: Glycosylation results with different (6′–6)-tethered glucose residues.



Entry	Linker (X)	Yield [%]	α/β
1		37	89:11
2		67	93:7
3		86	99:1
4		77	3:97

6.3. Linkage of the Glycosyl Donor and Acceptor through Nonreacting Centers.

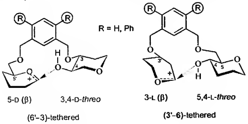
Glycosyl transfer within the active site of an enzyme can be regarded as an intramolecular process in which the glycosyl donor and the acceptor are held in close proximity to enforce regio- and stereoselective glycoside-bond formation.[290,293,299] To mimic this process in vitro, a system was designed in which the acceptor is attached to the donor through a spacer connected to nonreacting centers (Figure 4c). Particularly rigid spacers, which force the reacting centers into close proximity, should result in efficient glycoside-bond formation.[290] In this way, the spacer remains part of the target molecule and has to be removed in a second step; therefore, intramolecular product formation is evident. Various terms have been employed for this very successful approach to oligosaccharide synthesis: "rigid spacer concept", "intramolecular glycosylation of prearranged glycosides", "template-directed cyclo-glycosylation", "remote glycosylation".[44]

Glycosylation reactions were investigated with a succinyl spacer between the donor and the acceptor to bring them into a "prearranged" position for high anomeric stereocontrol (transformation of **229** into **230**, Table 4, entry 2).[300] This spacer provides high conformational flexibility; therefore, the reaction centers are statistically too far from one another for the exclusive formation of one product. Surprisingly, this concept of a flexible succinyl spacer, and also the use of glutaryl and malonyl spacers, led to good to excellent stereocontrol and often good yields (Table 4, entries 1 and 2).[301] This approach was recently applied highly successfully to a glycosphingolipid synthesis.[302] Related investigations with peptide spacers containing asparagine residues at the N and C termini were not as successful, presumably as a result of interference by the amide groups.[303]

The "rigid spacer concept" was designed to bring the glycosyl donor and the acceptor into closer proximity.[290,300,304] As this approach leads to structurally more rigid molecules, a highly diastereoselective glycosylation should take place with the construction of a large ring. The *m*-xylylene group was chosen as an example of a rigid spacer, and 4,6-substitution restricted the conformational space of the glycosyl donor and acceptor even further (Table 5).[304] The ether linkages preclude any potential neighboring-group participation. Thus, the stereoselectivity of the intramolecular glycosylation reaction should be controlled through the relative orientation of the donor and acceptor moieties by the tethering spacer. The attachment site of the spacer on the donor (α or β site), the configuration of the acceptor (D,L-*threo* or D,L-*erythro*) within the macrocyclic ring, and the ring size should have a major influence. The results show that the stereoselectivity of the glycosylation is indeed controlled by the ring size (14- or 15-membered), by the configuration of the donor and of the two stereogenic centers of the acceptor (L-*threo*, L-*erythro*, D-*threo*, D-*erythro*) within the macrocyclic ring, and by the available conformational space.

Phthaloyl and isophthaloyl spacers can also be used to link donors with acceptors through nonreacting centers (Table 4, entry 3).[305] The replacement of these spacers with the di-*tert*-butylsilylene spacer led to a change in the preferred anomeric

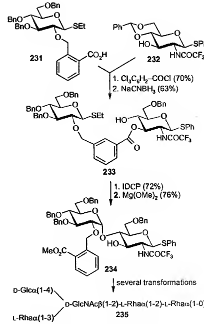
Table 5: Compilation of some results with an *m*-xylylene spacer.



Entry	Tethering	Donor config.	Acceptor config.	Ring size	Glycoside bond
1	6'–6	5-D (β)	5,4-L-*threo*	15	Glcβ(1-4)Glc
2	6'–4	5-D (β)	4,5-L-*threo*	15	Glcβ(1-6)Glc
3	6'–2	5-D (β)	2,3-L-*threo*	14	Glcβ(1-3)Glc
4	6'–4	5-D (β)	4,3-L-*erythro*	14	Glcβ(1-3)Gal
5	6'–3	5-D (β)	3,4-D-*threo*	14	Glcβ(1-4)Glc
6	6'–3	5-D (β)	3,4-D-*erythro*	14	Glcα(1-4)Gal
7	3'–6	3-L (β)	5,4-L-*threo*	14	Glcα(1-4)Glc

configuration as a result of the steric demand of the *tert*-butyl groups and the smaller ring size (Table 4, entry 4).[301] The combination of the *m*-xylylene and the isophthaloyl spacer led to nonsymmetric spacers, such as the 1,3-phenylene-1-carbonylmethyl spacer, which was employed for iterative intramolecular glycosylation reactions[306] and a very successful synthesis of the repeating unit of *Shigella flexneri* serotype 1a (Scheme 33, synthesis of the intermediate 235).[307]

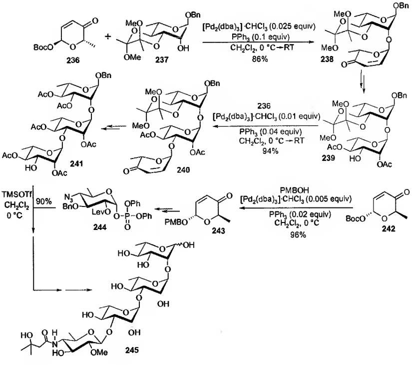
In the last 10–15 years, much effort has been devoted to the development of intramolecular glycosylation reactions as high-yielding processes with high anomeric stereoselectivity. Good solutions have been presented for the formation of nearly all important glycosidic linkages, including the generation of 1,2-*cis* glycosides as present in β-mannopyranosides and α-glucopyranosides. However, only very few applications to the synthesis of complex glycoconjugates have been reported so far. For intramolecular glycosylation to gain the general acceptance enjoyed by the intermolecular methods, further effort is needed to improve access to the required starting materials and to extend the method to the synthesis of complex glycoconjugates, ideally by simple iterative methods. Molecular modeling may assist in the selection of the appropriate spacer and attachment sites on the glycosyl donor and acceptor.



Scheme 33. Synthesis of the repeating unit of *Shigella flexneri* serotype 1a.

7. Other Aspects

Besides one-pot glycosylation and SPOS, many other techniques have been developed in recent years to expedite oligosaccharide synthesis. For example, a novel fluorous support was developed as an alternative to traditional polymer supports and applied successfully to oligosaccharide synthesis in combination with the trichloroacetimidate method.[308] Each intermediate in this oligosaccharide synthesis[309] could be isolated by partitioning between a fluorous solvent and an organic solvent, and the reactions could be monitored by TLC, NMR spectroscopy, and MS, in contrast to solid-phase reactions. Moreover, it is anticipated that the new liquid-phase technique will be readily applicable to large-scale synthesis.



Scheme 34. De novo asymmetric synthesis of the anthrax tetrasaccharide.

Much effort has also been directed towards a conceptually different glycosylation method, namely, the de novo synthesis of glycosides, although this method was investigated extensively and successfully many years ago.[310] In recent studies,[311] the glycoside bonds were constructed through palladium-catalyzed allylation, and the anomeric configuration could be controlled by the reagent rather than by anomeric or neighboring-group effects. The glycosylation usually proceeded in a highly stereoselective manner without the use of Lewis acid promoters, and the products could be elaborated readily to furnish natural or non-natural carbohydrates. By this approach, O'Doherty and co-workers synthesized a number of complex oligosaccharides and glycoconjugates.[312] The key steps in their new synthetic route to the anthrax tetrasaccharide **245** involved palladium-catalyzed glycosylation reactions (Scheme 34, synthesis of intermediates **238**, **240**, and **243**).[312h] The anthrax tetrasaccharide has also been synthesized by other procedures.[313]

8. Conclusions and Outlook

Undoubtedly, the advances in glycoside synthesis summarized herein have addressed some major problems associated with glycoside-bond formation and provided efficient strategies and powerful tools for accessing complex oligosaccharides and glycoconjugates of biological significance. However, one should bear in mind that carbohydrates and glycoconjugates are amongst the most complex biopolymers in nature. Their synthesis is still by no means routine and thus not comparable with peptide synthesis on the basis of amide-bond formation or oligonucleotide synthesis on the basis of phosphate diesters. Even for the construction of a simple glycosidic bond, careful optimization of all parameters, including the leaving group, promoter/catalyst, protecting groups, and glycosidation conditions, is often crucial for the reaction to proceed in high yield with high stereoselectivity. Hence, new conceptual approaches to glycosylation and novel strategies for the construction of complex oligosaccharides and glycoconjugates are still welcome to meet the intrinsic structural diversity of carbohydrates.

From where will this innovation come? Further variation of the leaving groups will probably not lead to major improvement of the existing methodologies for glycoside-bond formation. Rather, a deeper understanding of underlying mechanistic principles (ion-pair generation, memory effects of tight ion pairs, conformation-dependent reactivity, stereodifferentiation of the glycosyl donor between nucleophiles, and other factors) will lead to further advances. Interst in the use of enzymes, that is, glycosyltransferases, transglycosidases, and glycosidases, and manipulations based on their molecular biology (not discussed in this Review) may increase, particularly for the synthesis of specific glycosidic linkages and/or target molecules. Increased understanding of enzyme catalysis will also inspire new general concepts for the chemical regio- and stereoselective formation of glycoside bonds with minimization of the required protecting-group array, as is evident from methods already developed for intramolecular glycosidation. Such methods have been used to construct many glycoside bonds with excellent regio- and stereoselectivity.

Abbreviations

Ac	acetyl
ACB	2′-(allyloxycarbonyl)benzyl
ADMB	4-acetoxy-2,2-dimethylbutanoyl
All	allyl
BCB	2′-(benzyloxycarbonyl)benzyl
Bn	benzyl
Boc	*tert*-butoxycarbonyl
BSM	4-benzenesulfinylmorpholine
BSP	1-benzenesulfinylpiperidine
Bz	benzoyl
CA	chloroacetyl
CB	2′-carboxybenzyl
dba	dibenzylideneacetone
DBMP	2,6-di-*tert*-butyl-4-methylpyridine
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N′*-dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DMAP	4-dimethylaminopyridine
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	diisopropylethylamine
DME	dimethoxyethane
DMM	dimethylmaleoyl
DMTST	dimethyl(methylthio)sulfonium triflate
DPM	diphenylmethyl
dppf	1,1′-bis(diphenylphosphanyl)ferrocene
DPS	diphenyl sulfoxide
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
DTBS	di-*tert*-butylsilylene
EtSNPhth	*N*-(ethylthio)phthalimide
EWG	electron-withdrawing group
Fmoc	9-fluorenylmethoxycarbonyl
IAD	internal aglycone delivery
IDCP	iodonium dicollidine perchlorate
IPy_2BF_4	bis(pyridine)iodonium tetrafluoroborate
KHMDS	potassium hexamethyldisilazide
Lev	levulinoyl
MP	*p*-methoxyphenyl
NAP	2-napthylmethyl
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMM	*N*-methylmorpholine
NPG	*n*-pentenyl glycoside
NPOE	*n*-pentenyl orthoester
PA	phenoxyacetyl
PFP	pentafluoropropionyl
Phth	phthalimido
Piv	pivaloyl
PMB	*p*-methoxybenzyl
PTFA	*N*-phenyl trifluoroacetimidate
RDAS	reciprocal donor–acceptor selectivity
SBox	*S*-benzoxazolyl
SPOS	solid-phase oligosaccharide synthesis
STaz	*S*-thiazolyl

TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBD	1,5,7-triazabicyclo[4.4.0]dec-5-ene
TBS	*tert*-butyldimethylsilyl
TCA	trichloroacetyl
TCP	*N*-tetrachlorophthalimido
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TIPDS	1,1,3,3-tetraisopropyldisiloxane
TMS	trimethylsilyl
TMSI	trimethylsilyl iodide
Tol	toluene
Tr	trityl
triphos	1,1,1-tris(diphenylphosphanylmethyl)-ethane
Troc	2,2,2-trichloroethoxycarbonyl
TsOH	*p*-toluenesulfonic acid
TTBP	2,4,6-tri-*tert*-butylpyrimidine
UCP	unichemo protection

We apologize to all those researchers whose excellent work on glycoside-bond formation, particularly on applications to the synthesis of complex oligosaccharides and glycoconjugates, we could not cite owing to the focus of this Review. X.Z. thanks the University College Dublin and the Science Foundation Ireland for supporting his research. R.R.S. gratefully acknowledges financial support of his research by the University of Konstanz, the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, and the Alexander von Humboldt Foundation. Both authors thank their collaborators, particularly those mentioned in the references, for their valuable contributions to this field.

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Synthesis of β-(1→6)-Branched β-(1→3) Glucohexaose and Its Analogues Containing an α-(1→3) Linked Bond with Antitumor Activity

Jun Ning,[a,*] Wenhui Zhang,[a] Yuetao Yi,[a] Guangbin Yang,[a] Zhikui Wu,[b] Jie Yi[b] and Fanzuo Kong[a,*]

[a]*Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, PO Box 2871, Beijing 100085, PR China*
[b]*Guang Anmen Hospitol, Chinese Academy of Traditional Chinese Medicine, PR China*

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Abstract—A β-(1→6)-branched β-(1→3)-glucohexaose, present in many biologically active polysaccharides from traditionally herbal medicines such as *Ganoderma lucidum*, *Schizophyllum commune* and *Lentinus edodes*, was synthesized as its lauryl glycoside **32**, and its analogues **18**, **20** and **33** containing an α-(1→3) linked bond were synthesized. It is interesting to find that coupling of a 3,6-branched acylated trisaccharide trichloroacetimidate donor **9** with 3,6-branched acceptors **13** and **16** with 3′-OH gave the α-(1→3)-linked hexasaccharides **17** and **19**, respectively, in spite of the presence of C-2 ester capable of neighboring group participation. However, coupling of **9** with 4-methoxyphenyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**27**) selectively gave β-(1→3)-linked tetrasaccharide **28**. Simple chemical transformation of the tetrasaccharide **28** gave acylated tetrasaccharide trichloroacetimidate **29**. Coupling of **29** with lauryl (1→6)-linked disaccharide **26** with 3-OH gave β-(1→3)-linked hexasaccharide **30** as the major product. Bioassay showed that in combination with the chemotherapeutic agent cyclophospamide (CPA), the hexaose **18** at a dose of 0.5–1 mg/kg substantially increased the inhibition of S_{180} for CPA, but decreased the toxicity caused by CPA. Some of these oligosaccharides also inhibited U_{14} noumenal tumor in mice effectively.

Introduction

β-(1→6)-Branched β-(1→3) gluco-oligosaccharides are the common structure characteristic of many biologically active polysaccharides from traditionally herbal medicines such as *Ganoderma lucidum, Schizophyllum commune* and *Lentinus edodes.*[1] Of them, polysaccharides from *Lentinus edodes* called lentinan have the most strong antitumor effect, particularly, for Sarcoma-180 in mice, and have been used as an antitumor and immune reinforce medicine in Japan and China for many years. Clinically, lentinan has proved effective with chemotherapeutic agents for patients with recurrent gastric and colorectal cancer.[2] Some physicochemical and immunopharmacological investigations showed that the antitumor activity of these glucans may be closely related to the triplet-helix structures of the β-(1→3)-linked backbone chains[3a,b] and some biological aspects of β-glucans were reported.[3c,d,e] It was also reported that only higher molecular-weight fractions (MW >16,000) obtained from partial hydrolysis of lentinan with formic acid showed antitumor activity.[4] We hypothesize that the activity of lentinan is dependent upon its basic structure-oligosaccharide unit rather than its macroscopical morphology. It was reported that the β-(1→3)-branched β-(1→6) polysaccharides (MW $\sim 10^6$) of mycelial walls of the fungus *Phytophthora megasperma* f. sp. *Glycinea* can induce the formation of phytoalexins in soybean, and the heptasaccharide β-D-Glc*p*-(1→6)-[β-D-Glc*p*-(1→3)-]β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-[β-D-Glc*p*-(1→3)-]β-D-Glc*p*-(1→6)-D-Glc*p* and the hexasaccharide β-D-Glc*p*-(1→6)-[β-D-Glc*p*-(1→3)-]β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-[β-D-Glc*p*-(1→3)-]D-Glc*p* present in the polysaccharides have even more strong activity than the polysaccharides themselves.[5] Therefore, we deduce that the fragment of lentinan β-D-Glc*p*-(1→3)-[β-D-Glc*p*-(1→6)-]β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-[β-D-Glc*p*-(1→6)-]β-D-Glc*p*-(1→3)-D-Glc*p* or β-D-Glc*p*-(1→3)-[β-D-Glc*p*-(1→6)-]β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-[β-D-Glc*p*-(1→6)-]D-Glc*p* may have similar or better biological activities compared to lentinan itself.

*Corresponding author. Tel.: +86-10-62849157; fax: +86-10-62923563; e-mail: fzkong@mail.rcees.ac.cn

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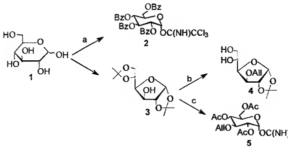
Here we would like to disclose the synthesis and biological activity of lentinan hexasaccharide and its analogues with an α-linkage in the backbone.

Results and Discussion

Preparation of oligosaccharides is more difficult and complex compared to the synthesis of other biopolymers such as peptides and nucleic acids. Most glycosylation methods are extremely sensitive to structural variations in the glycosyl donor-acceptor pairs.[6] Reaction conditions that provide excellent yields with one donor–acceptor pair may give virtually no product for another donor–acceptor pair. Furthermore, the stereochemical outcome is often difficult to predict. Our synthesis of the β-(1→6)-branched β-(1→3) glucohexaose again showed complexity of the oligosaccharide synthesis as described below.

More than 3 years ago we invented an efficient method for the synthesis of 3,6-branched β-linked gluco-oligosaccharids using 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose as the starting glycosyl acceptor.[7] The synthesis of β-(1→3)-branched β-(1→6)-linked glucohexaose phytoalexin elicitor on a 100 g scale was achieved in our laboratory and higher oligosaccharides of the elicitor including the hepta-, nona-, dodeca- and tetradecasaccharids were also readily synthesized by the developed strategy.[8] However, when the same strategy was used to prepare the β-(1→6)-branched β-(1→3)-linked glucohexaoses from a trisaccharide donor and a trisaccharide acceptor, the desired β-linked target was not obtained, but α-(1→3)-linked analogue was the product.[9,10]

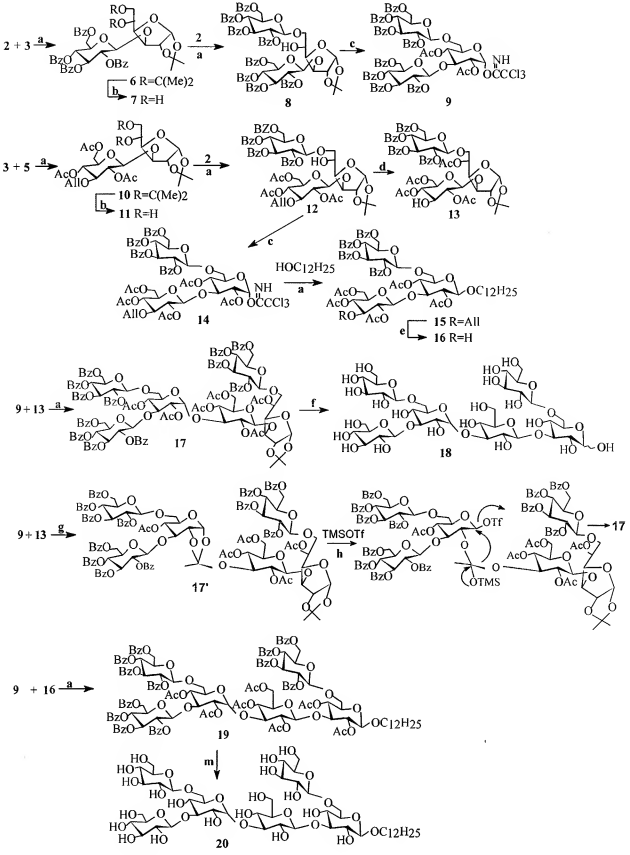
In our synthesis, 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate **2**, 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **3**, 3-*O*-allyl-1,2-*O*-isopropylidene-α-D-glucofuranose **4** and 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl trichloroacetimidate **5** were the starting materials and the trisaccharides **8**, **12** and the disaccharide **21** were the key intermediates. Compound **2** was prepared as fine crystals via benzoylation of D-glucose followed by 1-*O*-debenzoylation with ammonia in THF–CH_3OH and trichloroacetimidation (Scheme 1). Compound **3** was readily obtained by diacetonation of D-glucose according the standard method. Compound **4** was prepared from allylation of **3** followed by selective removal of 5,6-*O*-isopropylidation with 90% HOAc. Compound **5** was obtained as white crystals from the allylation of **3** followed by deisopropylidenation, acetylation, 1-*O*-deacetylation and trichloroacetimidation. The coupling of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **3** with perbenzoyl glucosyl trichloroacetimidate **2** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the catalyst, followed by selective 5,6-*O*-deacetonation afforded β-(1→3)-linked disaccharide **7** as crystals in high yield (81% over the two steps, Scheme 2).



Scheme 1. Keys: (a) (i) PhCOCl, pyridine and toluene, 70 °C, 8 h; (ii) 3:1 (v/v) THF–CH_3OH, 1.5 N NH_3, rt, 12 h; (iii) CH_2Cl_2, CCl_3CN and K_2CO_3, rt, 24 h. (b) (i) AllBr, NaH and DMF, rt, 2h; (ii) 90% HOAc, 40 °C, 24 h. (c) (i) AllBr, NaH and DMF, rt, 2h; (ii) 80% HOAc, reflux, 4h; (iii) Ac_2O–Pyridine, rt, 2h; (iv) 3:1 (v/v) THF–CH_3OH, 1.5 N NH_3, rt, 3 h; (v) CH_2Cl_2, CCl_3CN and K_2CO_3, rt, 24 h.

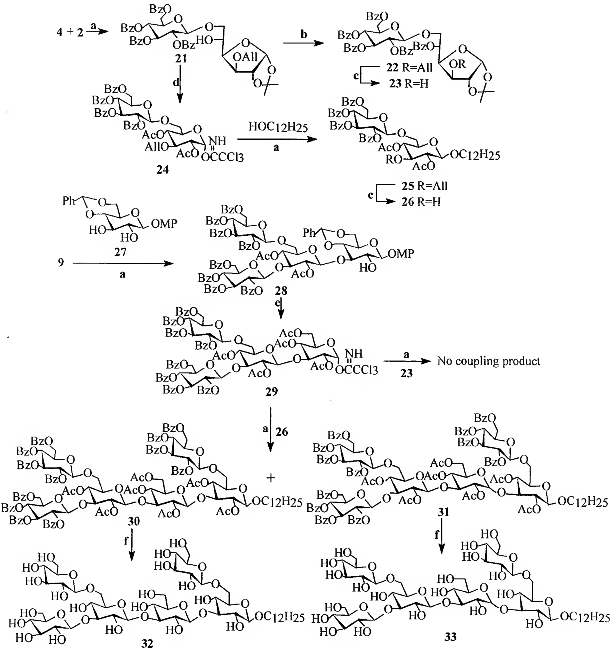
Condensation of **7** with **2** catalyzed by TMSOTf, regio- and stereoselectively gave one of the key intermediates: the 3,6-branched trisaccharides **8** in excellent yield (90%). Removal of the 1,2-*O*-isopropylidene of **8** in 80% HOAc followed by acetylation with acetic anhydride in pyridine, selective 1-*O*-deacetylation with ammonia in THF–CH_3OH, and subsequent treatment with trichloroacetonitrile in the presence of K_2CO_3 afforded the trisaccharide glycosyl donor **9** in good yield (71% over the four steps). Using the same procedure as described for the preparation of **8**, another key trisaccharide **12** was obtained from compounds **5**, **3**, and **2**. Acetylation of **12** with acetic anhydride in pyridine followed by deallylation with $PdCl_2$ in CH_3OH–CH_2Cl_2 afforded the trisaccharide glycosyl acceptor **13** in high yield (85%). Under the same conditions as described for the synthesis of **9** from **8**, compound **14** was obtained in good yield (71% over the four steps) from **12**. Coupling of **14** with $C_{12}H_{25}OH$ followed by deallylation afforded another trisaccharide glycosyl acceptor **16** in good yield (67% over the two steps). Coupling of the trisaccharide glycosyl donor **9** with either trisaccharide glycosyl acceptor **13** or **16**, catalyzed by TMSOTf in CH_2Cl_2, did not afford the expected β-linked haxasaccharides, but gave the pure α-linked haxasaccharides **17** and **19**, respectively, in excellent yields (86% for **17**, 84% for **19**). In the synthesis, the coupling of **9** with **13** gave an orthoester intermediate **17′** that was isolated and well identified, and could be transformed to **17** in the presence of catalytic TMSOTf. Deisopropylidenation of **17** in 80% HOAc, followed by deacylation in an ammonia-saturated solution in 1:1 CH_2Cl_2–CH_3OH, furnished the free hexasaccharide **18** as an amorphous white solid in 92% yield (over the two steps), and deacylation of **19** gave **20** as an amorphous white solid in 95% yield.

During our research, we found that the stereo-selectivity for the formation of (1→3) linked glucosyl bonds from the donor with C2 ester capable of neighboring group participation is influenced by the structure of both the glycosyl acceptors and glycosyl donors and some of these research results have been published recently.[11] For the synthesis of fully β-linked oligosaccharides, 4,6-*O*-benzylidene-β-D-glucopyranose was used as the acceptor since its derivatives often give the β-(1→3) linked glucosyl bonds exclusively as reported.[12] Thus condensation of **2** with **4**, catalyzed by TMSOTf, regioselectively gave the key disaccharide **21** in excellent yield



Scheme 2. Keys: (a) TMSOTf, 4 Å MS. CH_2Cl_2, rt, 3 h. (b) 90% HOAc, 40 °C, 24 h. (c) (i) 80% HOAc, reflux, 5 h; (ii) Ac_2O–Pyridine, rt, 2 h; (iii) THF–CH_3OH, 1.5 N NH_3, rt, 3 h; (iv) CH_2Cl_2, CCl_3CN and K_2CO_3, rt, 24 h. (d) (i) Ac_2O–Pyridine, rt, 2 h; (ii) $PdCl_2$, CH_2Cl_2–CH_3OH, rt, 5 h. (e) $PdCl_2$ in CH_2Cl_2–CH_3OH, rt, 5 h. (f) (i) 80% HOAc, reflux, 6 h; (ii) CH_2Cl_2–CH_3OH saturated with ammonia, rt, 24 h. (g) TMSOTf, 4 Å MS, CH_2Cl_2, rt, 20 min. (h) TMSOTf, 4 Å MS, CH_2Cl_2, rt, 3 h. (m) CH_2Cl_2–CH_3OH saturated with ammonia, rt, 24 h.

(84%) (Scheme 3). The disaccharide glycosyl acceptor **23** was subsequently obtained by 5-*O*-benzoylation of **21** followed by 3-*O*-deallylation in 78% yield. Lauryl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,4-di-*O*-acetyl-β-D-glucopyranoside (**26**) as another disaccharide glycosyl acceptor was prepared from condensation of **24** with lauryl alcohol followed by deallylation. Regio- and stereoselective coupling of **9** with 4-methoxyphenyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**27**)[13] afforded the desired tetrasaccharide **28** (77% yield) which was then converted to the tetrasaccharide glycosyl donor **29** by removal of the 4,6-*O*-benzylidene of **28** in 90% HOAc followed by acetylation, demethoxyphenylation with $(NH_4)_2Ce(NO_3)_6$ in CH_3CN-H_2O,[14] and subsequent trichloroacetimidation in 71% yield (over the four steps). However, coupling of **29** with **23**, catalyzed by TMSOTf in CH_2Cl_2, gave nothing except the decomposed product of glycosyl donor **29** and the unchanged glycosyl acceptor **23**. Condensation of **29** with **26** afforded a mixture of the β-(1→6)-branched β-(1→3)-linked glucohexatose **30** (38% yield) and its isomer containing an α-(1→3) linked bond **31** (16% yield). At the initial stage of the coupling, an orthoester intermediate **30′** was obtained and could be isolated. With addition of the extra catalyst TMSOTf or elongation of the reaction time, **30′** was transformed to a mixture of **30**



Scheme 3. Keys: (a) TMSOTf, 4 Å MS, CH_2Cl_2, rt, 3 h. (b) PhCOCl, pyridine, rt, 2 h. (c) $PdCl_2$ in CH_2Cl_2–CH_3OH, rt, 3 h. (d) (i) 80% HOAc, reflux, 5 h; (ii) Ac_2O–Pyridine, rt, 2 h; (iii) THF–CH_3OH, 1.5 N NH_3, rt, 3 h; (iv) CH_2Cl_2, CCl_3CN and K_2CO_3, rt, 24 h. (e) (i) 90% HOAc, 40 °C, 24 h; (ii) Ac_2O–Pyridine, rt, 2 h; (iii) $(NH_4)_2Ce(NO_3)_6$ in CH_3CN–H_2O, rt, 0.5 h; (iv) CH_2Cl_2, CCl_3CN and K_2CO_3, rt, 24 h. (f) CH_2Cl_2–CH_3OH saturated with ammonia, rt, 24 h.

and **31**. It seemed that transformation of the orthoester **30′** to the corresponding oligosaccharides **31** and **32** went through two different paths as indicated in Scheme 4. Path 1 (→**30**) was the normal rearrangement leading to trans glycosylation, while path 2 (→**31**) was the unusual one caused by C1-O1 breaking of the orthoester.[11] Finally, compounds **32** and **33** were obtained by deprotection of **30** and **31**, respectively.

Bioassay showed that in combination with the chemotherapeutic agent cyclophospamide (CPA), the hexaose **18** at a dose of 0.5–1 mg/kg subatantially increased the inhibition of CPA to S_{180}, but decreased the toxicity caused by CPA as indicated from the amount of nucleated cell in bone marrow (Table 1).

Inhibition of U_{14} noumenal tumor by the synthetic olgosaccharides was also investigated indicating that the allyl heptaoside, a fully β-linked lentinan repeating unit,[13] and lauryl hexaoside **20** were potential therapeutic agents for cancer treatment (Table 2).

Now in our laboratory, a lot of derivatives of the (1→6)-branched (1→3)-linked glucohexaoses are in preparation, and interesting results about the structure–activity relationship of the newly discovered biologically active oligosaccharides including **32** and **33** will be reported in due course.

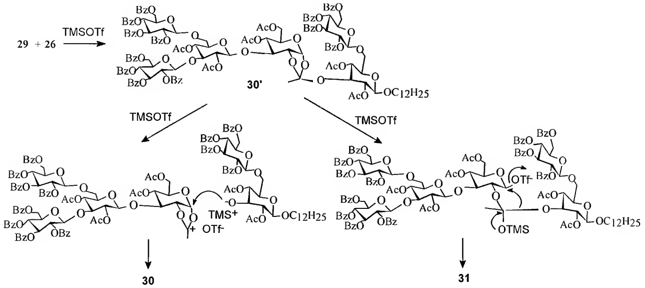
Table 2. Inhibition of U_{14} tumor by **18**, **20**, allyl heptaoside, and CPA

Groups	Weight (g)	Quotient of noumenal tumor (%)	Inhibition (%)
U_{14} cell line group	29.33 ± 1.65	4.21 ± 1.91	
Group 1: treatment with **18** (10 mg/kg)	27.40 ± 1.90	2.63 ± 0.90	29.41
Group 2: treatment with **20** (5 mg/kg)	27.45 ± 2.67	1.54 ± 0.56**	58.43
Group 3: treatment with allyl heptaoside (5 mg/kg)	28.10 ± 1.79	1.51 ± 0.41**	58.43
Group 4: treatment with CPA (70 mg/kg)	28.00 ± 1.78	1.86 ± 0.73*	39.22

Compared to U_{14} cell line group, *$p < 0.05$ **$p < 0.01$.

Experimental

Melting points were determined with a 'Mel-Temp' apparatus. Optical rotations were determined at 25 °C with digital polarimeter. The NMR spectra were recorded in $CDCl_3$ with TMS internal standard or D_2O with ethanol as standard on ARX 400 MHz. Mass spectra were recorded on an autospec mass spectrometer using ESI technique to introduce the sample. Elemental analyses were done on elemental analyzer model 1108 EA. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/



Scheme 4.

Table 1. Inhibition of S_{180} and effect on nucleated cell in bone marrow for the hexaose **18** and **18** + CPA

Groups	Weight (g)	Amount of nucleated cell (× 10^7)	Amount of tumor cell (× 10^7)
Control group	22.29 ± 0.95	7.45 ± 2.35###	
S_{180} Cell line group	22.00 ± 1.00	7.65 ± 3.05	2.94 ± 0.54
Group 1: treatment with **18** (1 mg/kg)	21.86 ± 0.90	6.70 ± 1.95###∧	2.01 ± 0.56*
Group 2: treatment with **18** (1 mg/kg) + CPA (50 mg/kg)	22.00 ± 1.00	3.60 ± 1.15#	1.53 ± 0.85***
Group 3: treatment with **18** (0.5 mg/kg)	21.71 ± 0.95	6.80 ± 2.00###∧	2.38 ± 0.61*
Group 4: treatment with **18** (0.5 mg/kg) + CPA (50 mg/kg)	22.00 ± 0.82	3.55 ± 1.55##	1.57 ± 0.62**
Group 5 treatment with CPA (50 mg/kg)	21.86 ± 0.69	1.50 ± 0.45##	2.01 ± 0.53*

Compared to group 5, #$p < 0.05$. ##$p < 0.01$. ###$p < 0.001$. Comparison of a kind of drug to the combined two, ∧$p < 0.05$. *Compared to the S_{180} cell line group, $p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

v) H_2SO_4 in MeOH or in some cases by a UV detector. Column chromatography was conducted by elution of a column (10×240 mm, 18×300 mm, 35×400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. Solutions were concentrated at <60 °C under diminished pressure. Dry solvents were distilled over CaH_2 and stored over molecular sieves.

Bioassay for inhibition of S_{180}

Cyclophosphamide was purchased from Shanghai Hualian Pharmaceutical Co. Ltd. (permission No. Hu Medicines 95-012034). Animals: 50 Kunming male mice (weight 20±2 g, grade II) were obtained from the Experimental Animal Center, the Chinese Academy of Medicines. S_{180} cell line was provided by the Department of Tumorology, Institute of Pharmacy, the Chinese Academy of Medicines. The mice were randomly divided into seven groups according to weight, and into each of them was planted S_{180} cell line of ascites cancer 0.2 mL (amount of cell 7.9×10^6) in their euterocelia by intraperitoneal except for the control group mice. At 24 h after the planting, the mice were treated with the synthetic oligosaccharides or cyclophosphamide. The oligosaccharide was dissolved in saline at 0.1 mg/mL, given with a dose of 0.5 or 1 mg/kg/day to the mice within three consecutive days. Cyclophosphamide was dissolved in saline at 4 mg/mL, and given to the mice at a dose of 20 mg/kg by intraperitoneal at 24 h after the planting, and 30 mg/kg at 48 h after the planting. For inhibition of U_{14}, the same mice were used and divided into seven groups. Into each of the mice was planted U_{14} cell line 0.2 mL (amount of cell 4×10^6) by intraperitoneal. At 24 h after the planting, the mice were treated with the synthetic oligosaccharides or cyclophosphamide. The oligosaccharides **18** and **20** were dissolved in saline at 1 mg/mL, given with a dose of 10 mg/kg/d to the mice within ten consecutive days (0.2 mL/day, ip). Allyl heptaoside was dissolved in saline at 1 mg/mL, given with a dose of 5 mg/kg/day to the mice within ten consecutive days (0.1 mL/day ip). Cyclophosphamide was dissolved in saline at 6 mg/mL, and given to the mice at a dose of 30 mg/kg by intraperitoneal at 24 h after the planting, and 20 mg/kg at 72 h and 120 h after the planting, respectively.

2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (2). BzCl (68 mL, 583 mmol) was added to a solution of D-glucose (**1**) (20 g, 111 mmol) in toluene (250 mL) and pyridine (47.3 mL, 585 mmol) over 1 h and the temperature was kept at 70 °C, and then the mixture was stirred at 70 °C for further 7 h. Filtration of pyridinum hydrochloride salt and concentration of the filtrate gave a residue which was directly dissolved in a 1.5 N solution of NH_3 in THF (350 mL) and CH_3OH (210 mL). The solution was kept at room temperature for 12 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, and the residue was dissolved in a solution of CH_2Cl_2 (100 mL) and CCl_3CN (12 mL, 120 mmol) containing K_2CO_3 (30 g, 217 mmol). The reaction mixture was stirred for 24 h at rt, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtrated, the solution was concentrated under reduced pressure, and the residue was decolorized by passing through a short silica-gel column with 3:1 petroleum ether–EtOAc as the eluent and 2,3,4,6-tetra-*O*-benzyol-α-D-glucopyranosyl trichloroacetimidate (**2**) (46 g, 56% for three steps) was crystallized from 3:1 petroleum ether–EtOAc as white crystals.[15]

3-*O*-Allyl-1,2-*O*-isopropylidene-α-D-glucopyranose (4). To a solution of **3** (10.0 g, 38.4 mmol) in dry DMF (50 mL), AllBr (3.7 mL, 42.2 mmol) and NaH (3.0 g, 49% in oil, 61.4 mmol) were added under cooling with an ice bath. The mixture was stirred for 2 h at rt, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was poured to water and extracted with CH_2Cl_2. The organic phase was concentrated, and the resulting residue was directly dissolved in 90% acetic acid solution (80 mL). The mixture was kept at 40 °C for 24 h and then concentrated to a residue under reduced pressure. The residue was purified by flash chromatography (2:1 petroleum ether–EtOAc) to give **4** (7.1 g, 71% for two steps) as syrup. $[\alpha]_D$ +14° (*c* 1.2, $CHCl_3$); ^{1}HNMR(400 MHz, $CDCl_3$) δ 5.92 (d, 1H, J=3.8 Hz), 5.90 (m, 1H), 5.32 (dd, 1H, J=1.3, 17.1 Hz), 5.23 (dd, 1H, J=1.3 Hz, 10.4 Hz), 4.58 (d, 1H, J=3.8 Hz), 4.22–4.03 (m, 5H), 3.84 (dd, 1H, J=3.4, 11.4 Hz), 3.74 (dd, 1H, J=5.5, 11.4 Hz). Anal. calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.74. Found: C, 55.81; H, 7.80.

3-*O*-Allyl-2,4,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (5). To a solution of **3** (50.0 g, 192 mmol) in dry DMF (160 mL), AllBr (18.6 mL, 211 mmol) and NaH (15.0 g, 49% in oil, 307 mmol) were added under cooling with an icc bath. The mixture was stirred for 2 h at rt, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was poured to water and extracted with CH_2Cl_2. The organic phase was concentrated, and the resulting residue was directly dissolved in 80% aqueous acetic acid solution (300 mL), and the mixture was heated under reflux for 4 h. The mixture was concentrated, and the residue was treated with acetic anhydride (250 mL) in pyridine (280 mL) for 2 h at rt. The acetylated sugar was dissolved in a 1.5 N solution of NH_3 in 3:1 THF–CH_3OH (300 mL), and the solution was kept at rt for 3 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, and the residue was dissolved in a solution of CH_2Cl_2 (200 mL) and CCl_3CN (39 mL, 384 mmol) containing K_2CO_3 (50 g, 362 mmol). The reaction mixture was stirred for 24 h at rt. After filtering the mixture, the filtration and washings were concentrated, and the residue was subjected to column chromatography with 3:1 petroleum ether–EtOAc as the eluent to give **5** (38.4 g, 54% for four steps) as a white crystals. Mp 71–73 °C; $[\alpha]_D$ +0.15° (*c* 1.5, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 6.52 (d, 1H, J=3.6 Hz), 5.80 (m, 1H), 5.24 (dd, 1H, J=1.3, 17.1 Hz), 5.18–5.13 (m, 2H), 5.03 (dd, J=3.6, 13.6 Hz), 4.24–4.18 (m, 2H), 4.13–4.08

(m, 3H), 3.97 (t, 1H, $J=9.6$ Hz), 2.10, 2.08, 2.07 (3 s, 9H). Anal. calcd for $C_{17}H_{22}NO_9Cl_3$: C, 55.13; H, 5.99. Found: C, 55.62; H, 5.90.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-1,2-*O*-isopropylidene-α-D-glucofuranose (7). To a stirred solution of **3** (10 g, 38 mmol) and **2** (26 g, 35 mmol) in CH_2Cl_2 (100 mL) was added TMSOTf (30 μL) at room temperature. After 3 h, triethylamine was added to the solution to quench the reaction. The solution was concentrated, the resulting residue was directly dissolved in 90% aqueous acetic acid solution (200 mL). The mixture was kept at 40 °C for 24 h and then concentrated to a residue under reduced pressure. The resulting residue was subjected to a short silica-gel column to give compound **7** (22.6 g, 81% for two steps). Mp 120–123 °C; $[\alpha]_D$ +14° (*c* 2.5, $CHCl_3$). ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.11–7.28 (m, 20H), 5.94 (t, 1H, $J=9.7$ Hz), 5.72 (t, 1H, $J=9.7$ Hz), 5.54 (dd, 1H, $J=7.9$, 9.7 Hz), 5.53 (d, 1H, $J=3.6$ Hz), 5.03 (d, 1H, $J=7.9$ Hz), 4.84 (dd, 1H, $J=3.6$, 11.9 Hz), 4.42 (dd, 1H, $J=4.3$, 11.9 Hz), 4.41(d, 1H, $J=2.6$ Hz), 4.24–4.23 (m, 2H), 4.16 (dd, 1H, $J=2.6$, 8.8 Hz), 4.02 (m, 1H), 3.83 (dd, 1H, $J=3.2$, 11.4 Hz), 3.67 (dd, 1H, $J=6.0$, 11.4 Hz), 1.44, 1.09 (2 s, 6H). Anal. calcd for $C_{43}H_{42}O_{15}$: C, 64.66; H, 5.30. Found: C, 64.94; H, 5.25.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-1,2-*O*-isopropylidene-α-D-glucofuranose (8). To a stirred solution of **7** (8 g, 10 mmol) and **2** (8 g, 10.8 mmol) in CH_2Cl_2 (60 mL) was added TMSOTf (30 μL) at room temperature. After 3 h, triethylamine was added to the solution to quench the reaction. The solution was concentrated, the residue was subjected to column chromatography with 1.5:1 petroleum ether–ethyl acetate as the eluent to give **8** (12.4 g, 90%). $[\alpha]_D$ +19.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06–7.28 (m, 40H), 5.88 (t, 1H, $J=9.7$ Hz), 5.87 (t, 1H, $J=9.7$ Hz), 5.69 (t, 1H, $J=9.7$ Hz), 5.64 (t, 1H, $J=9.7$ Hz), 5.53 (dd, 1H, $J=7.9$, 9.7 Hz), 5.43 (dd, 1H, $J=7.9$, 9.7 Hz), 5.41 (d, 1H, $J=3.5$ Hz), 4.96 (d, 1H, $J=7.9$ Hz), 4.93 (d, 1H, $J=7.9$ Hz), 4.71–4.67 (m, 2H), 4.48 (dd, 1H, $J=4.9$, 12.2 Hz), 4.35 (dd, 1H, $J=4.9$, 12.2 Hz), 4.34–3.65 (m, 8H), 1.26, 1.03 (2 s, 6H). Anal. calcd for $C_{77}H_{68}O_{24}$: C, 67.15; H, 4.98. Found: C, 66.89; H, 5.09.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (9). Compound **8** (10 g, 7.2 mmol) was added to 80% aqueous acetic acid solution (100 mL) and the mixture was heated under reflux for 5 h. The mixture was concentrated, and the residue was acetylated with acetic anhydride (20 mL) in pyridine (20 mL) for 2 h at rt. The resultant trisaccharide was dissolved in a 1.5 N solution of NH_3 in 3:1 THF–CH_3OH (100 mL), and the solution was kept at rt for 3 h. The solution was concentrated, the residue was dissolved in CH_2Cl_2 (50 mL). To the solution were added K_2CO_3 (2 g, 14.5 mmol) and CCl_3CN (1.4 mL, 14.4 mmol), and the mixture was stirred at rt for 24 h. Filtering the mixture, the filtration and washings were concentrated, and the residue was subjected to column chromatography to give **9** (8.0 g, 71% for four steps). $[\alpha]_D$ +23.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.07–7.19 (m, 40H), 6.21 (d, 1H, $J=3.6$), 5.91 (t, 1H, $J=9.6$ Hz), 5.85 (t, 1H, $J=9.6$ Hz), 5.62 (t, 1H, $J=9.6$ Hz), 5.61 (t, 1H, $J=9.6$ Hz), 5.46 (dd, 1H, $J=7.9$, 9.6 Hz), 5.42 (dd, 1H, $J=7.9$, 9.6 Hz), 4.97 (d, 1H, $J=7.9$ Hz), 4.96 (d, 1H, $J=7.9$ Hz), 4.85 (t, 1H, $J=9.5$ Hz), 4.67–4.59 (m, 3H), 4.50–4.37 (m, 2H), 4.19–4.02 (m, 4H), 3.91(dd, 1H), 3.69 (dd, 1H), 1.94, 1.78 (2 s, 6H). Anal. calcd for $C_{80}H_{68}NO_{26}Cl_3$: C, 61.37; H, 4.38. Found: C, 61.93; H, 4.51.

3-*O*-Allyl-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-1,2-*O*-isopropylidene-α-D-glucofuranose (11). To a stirred solution of **3** (10 g, 38.5 mmol) and **5** (14.1 g, 38.0 mmol) in CH_2Cl_2 (100 mL) was added TMSOTf (35 μL) at room temperature. After 3 h, triethylamine was added to the solution to quench the reaction. The solution was concentrated, the resulting residue was directly dissolved in 90% aqueous acetic acid solution (150 mL). The mixture was kept at 40 °C for 24 h and then concentrated to a residue under reduced pressure. The resulting residue was subjected to a short silica-gel column to give compound **11** (15 g, 72% for two steps) as a white crystals. Mp 91–93 °C; $[\alpha]_D$ +42 (*c* 1.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.77 (d, 1H, $J=3.6$ Hz), 5.71 (m, 1H), 5.14 (dd, 1H), 5.08 (dd, 1H), 4.96 (t, 1H, $J=9.6$ Hz), 4.90 (dd, 1H, $J=7.9$, 9.4 Hz), 4.53 (d, 1H, $J=7.9$ Hz), 4.30 (d, 1H), 4.22 (d, 1H), 4.12–4.00 (m, 5H), 3.87 (m, 1H), 3.77 (dd, 1H), 3.60–3.58 (m, 2H), 3.54 (t, 1H, $J=9.4$ Hz), 2.05, 2.03, 2.02 (3 s, 9H), 1.42, 1.25 (2 s, 6H). Anal. calcd for $C_{24}H_{36}O_{14}$: C, 52.55; H, 6.61. Found: C, 52.81; H, 6.52.

3-*O*-Allyl-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-1,2-*O*-isopropylidene-α-D-glucofuranose (12). To a stirred solution of **11** (20 g, 36.5 mmol) and **2** (27.4 g, 37 mmol) in CH_2Cl_2 (150 mL) was added TMSOTf (40 μL) at room temperature. After 3 h, triethylamine was added to the solution to quench the reaction. The solution was concentrated, the residue was subjected to column chromatography with 1.5:1 petroleum ether–ethyl acetate as the eluent to give the trisaccharide **12** (37.0 g, 90%). $[\alpha]_D$ +16.1 (*c* 1.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02–7.27 (m, 20H), 5.89 (t, 1H, $J=9.6$ Hz), 5.76 (d, 1H, $J=3.7$ Hz), 5.75 (m, 1H), 5.69 (t, 1H, $J=9.6$ Hz), 5.55 (dd, 1H, $J=7.9$, 9.6 Hz), 5.20 (dd, 1H), 5.14 (dd, 1H), 5.01 (d, 1H, $J=7.9$ Hz), 5.00 (t, 1H, $J=9.7$ Hz), 4.89 (dd, 1H, $J=7.9$, 9.3 Hz), 4.67 (dd, 1H), 4.52 (d, 1H, $J=7.9$ Hz), 4.48 (dd, 1H), 4.33 (d, 1H), 4.23 (d, 1H), 4.17–4.01 (m, 8H), 3.80 (m, 1H), 3.60–3.54 (m, 2H), 2.08, 2.07, 2.02 (3 s, 9H), 1.32, 1.26 (2 s, 6H). Anal. calcd for $C_{58}H_{62}O_{23}$: C, 61.81; H, 5.54. Found: C, 61.46; H, 5.61.

2,4,6-Tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (13). The compound **12** (25 g, 22.2 mmol) was acetylated with acetic anhydride (20 mL) in pyridine (20 mL) at rt for 2 h. The mixture was diluted with dichloromethane, washed with M HCl, water, and satd aq sodium bicarbonate subsequently.

The organic layer was combined, dried, and concentrated. The residue was dissolved in a solution of CH_3OH (250 mL) containing $PdCl_2$ (100 mg). The mixture was stirred for 5 h at rt, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, and the filtrate was concentrated, and the residue was passed a silica-gel column with 2:1 petroleum ether–EtOAc as the eluent to give **13** (21.3 g, 85%). $[\alpha]_D$ +26.6 (*c* 2.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.07–7.27 (m, 20H), 5.87 (t, 1H, J=9.5 Hz), 5.74 (t, 1H, J=9.7 Hz), 5.73 (d, 1H, J=3.5 Hz), 5.54 (dd, 1H, J=7.9, 9.5 Hz), 5.16 (m, 1H), 4.99 (t, 1H, J=9.6 Hz), 4.87 (d, 1H, J=7.9 Hz), 4.81 (dd, 1H, J=7.8, 9.3 Hz), 4.50 (d, 1H, J=7.8 Hz), 4.43–4.39 (m, 2H), 4.30–4.27 (m, 2H), 4.22–4.06 (m, 5H), 3.81 (m, 1H), 3.67 (t, 1H, J=9.6 Hz), 3.57 (m, 1H), 2.09, 2.08, 2.03, 1.73 (4 s, 12H), 1.37, 1.28 (2 s, 6H). Anal. calcd for $C_{57}H_{60}O_{24}$: C, 60.64; H, 5.36. Found: C, 60.98; H, 5.28.

3-*O*-Allyl-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (14). Compound **12** (14 g, 12.4 mmol) was added to 80% acetic acid solution (150 mL) and the mixture was heated under reflux for 5 h. The mixture was concentrated, and the residue was acetylated with acetic anhydride (20 mL) in pyridine (20 mL) for 2 h at rt. The mixture was diluted with dichloromethane, washed with M HCl, water, and satd aq sodium bicarbonate subsequently. The organic layer was combined, dried, and concentrated. The trisaccharide residue was dissolved in a 1.5 N solution of NH_3 in 3:1 THF–CH_3OH (150 mL), and the solution was kept at rt. After 3 h, the solution was concentrated, and the residue was dissolved in CH_2Cl_2 (80 mL). To the solution were added K_2CO_3 (5 g, 36.2 mmol) and CCl_3CN (2.0 mL, 20 mmol), and the mixture was stirred at rt for 24 h. Filtering the mixture, the filtration and washings were concentrated, and the residue was subjected to column chromatography to give the trisaccharide donor **14** (11.6 g, 71% for four steps). $[\alpha]_D$ +62.0 (*c* 1.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.04–7.26 (m, 20H), 6.22 (d, 1H, J=3.5 Hz), 5.86 (t, 1H, J=9.6 Hz), 5.71 (m, 1H), 5.64 (t, 1H, J=9.6 Hz), 5.47 (dd, 1H, J=7.8, 9.6 Hz), 5.18 (dd, 1H), 5.06 (dd, 1H), 5.00 (t, 1H, J=9.6 Hz), 4.94 (d, 1H, J=7.8 Hz), 4.87 (t, 1H, J=9.7 Hz), 4.86(t, 1H, J=9.6 Hz), 4.80(dd, 1H, J=7.9, 9.6 Hz), 4.62 (dd, 1H), 4.49 (d, 1H, J=7.9 Hz), 4.48 (dd, 1H), 4.22 (dd, 1H), 4.15–4.01 (m, 6H), 3.93 (d, 1H), 3.69–3.51 (m, 3H), 2.06, 2.05, 2.04, 2.01, 1.99 (5 s, 15H). Anal. calcd for $C_{61}H_{62}NO_{25}Cl_3$: C, 55.69; H, 4.75. Found: C, 55.19; H, 4.58.

Lauryl 3-*O*-allyl-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-glucopyranoside (15). To a solution of **14** (4.5 g, 3.42 mmol) and lauryl alcohol (0.93 g, 5.0 mmol) in CH_2Cl_2 (50 mL) was added TMSOTf (30 μL) at rt. The reaction mixture was stirred for 3 h, at the end of which time TLC indicated that the reaction was complete. Then the mixture was neutralized with triethylamine and concentrated under reduced pressure to dryness. Purification by column chromatography (3:1 petroleum ether–EtOAc) gave **15** (3.66 g, 80%) as a syrup: $[\alpha]_D$ +32.1° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.03–7.27 (m, 20H), 5.88 (t, 1H, J=9.8 Hz), 5.71 (m, 1H), 5.67 (t, 1H, J=9.2 Hz), 5.50 (dd, 1H, J=8.0, 9.6 Hz), 5.16 (dd, 1H), 5.11 (dd, 1H), 4,99 (t, 1H, J=9.2 Hz), 4.94 (d, 1H, J=8.0 Hz), 4.85 (d, 1H, J=8.0 Hz), 4.84 (d, 1H, J=8.0 Hz), 4.64 (m, 1H), 4.46 (m, 2H), 4.25 (dd, 1H, J=12.4, 3.2 Hz), 4.15–3.43 (m, 11H), 3.01 (m, 1H), 2.07, 2.06, 2.05, 2.04, 1.95 (5 s, 15H), 1.30–1.18 (m, 20H), 0.88 (t, 3H). Anal. calcd for $C_{71}H_{86}O_{25}$: C, 63.67; H, 6.47. Found: C, 64.01; H, 6.20.

Lauryl 2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-glucopyranoside (16). Compound **15** (3.4 g, 2.54 mmol) was dissolved in a solution of CH_3OH (70 mL) and CH_2Cl_2 (10 mL). To the solution was added $PdCl_2$ (25 mg), and the mixture was stirred for 5 h at rt, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, and the filtrate was concentrated, and the residue was passed through a silica-gel column with 2:1 petroleum ether–EtOAc as the eluent to give **16** (2.77 g, 84%). $[\alpha]_D$ +19.3 (*c* 2.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.03–7.27 (m, 20H), 5.88 (t, 1H, J=9.7 Hz), 5.67 (t, 1H, J=9.6 Hz), 5.50 (dd, 1H, J=7.9, 9.6 Hz), 4.93 (d, 1H, J=7.9 Hz), 4.89 (t, 1H, J=9.6 Hz), 4.87 (t, 1H, J=9.7 Hz), 4.70–4.62 (m, 2H), 4.47 (dd, 1H), 4.45 (d, 1H, J=7.9 Hz), 4.32 (dd, 1H), 4.16–4.14 (m, 2H), 4.03 (dd, 1H), 3.94 (d, 1H), 3.75 (t, 1H), 3.67–3.57 (m, 3H), 3.45 (m, 1H), 3.00 (m, 1H), 2.09, 2.08, 2.07, 2.06, 1.96 (5 s, 15H), 1.35–1.07 (m, 20H), 0.89 (t, 3H). Anal. calcd for $C_{68}H_{82}O_{25}$: C, 62.86; H, 6.36. Found: C, 62.41; H, 6.49.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-5-*O*-acetyl-1,2-*O*-isopropylidine-α-D-glucopyranoside (17). To a stirred solution of 9 (7.30 g, 4.66 mmol) and **13** (5.1 g, 4.5 mmol) in CH_2Cl_2 (60 mL) was added TMSOTf (30 μL) at rt. After 3 h, the mixture was neutralized with triethylamine and concentrated. The residue was purified on a silica gel column with 1.5:1 petroleum ether–EtOAc as the eluent to give **17** (9.8 g, 86%). $[\alpha]_D$ +18.7 (*c* 1.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.89–7.27 (m, 60H), 5.88, 5.87, 5.82, 5.75 (4 t, 4H, J=9.5 Hz), 5.72 (d, 1H, J=3.5 Hz), 5.68, 5.63 (2 t, 2H, J=9.5 Hz,), 5.59, 5.45, 5.38 (3 dd, 3H, J=7.9, 9.5 Hz,), 5.05 (m, 1H), 4.97, 4.92 (2 d, 2H, J=7.9 Hz), 4.90 (d, 1H, J=3.6 Hz), 4.84, 4.53 (2 d, 2H, J=7.9 Hz), 2.00, 1.97, 1.84, 1.83, 1.82, 1.77 (6 s, 18H), 1.37, 1.35 (2 s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.01, 169.81, 169.22, 168.93, 168.78, 168.31, 165.70, 165.62, 165.52, 165.40, 165.32, 165.17, 165.02, 164.75, 164.61, 164.54, 111.92, 104.74 (C-1 for α bond, $J_{C\text{-}H}$=182.4 Hz), 100.69, 100.62, 100.48, 97.76 (4 C-1 for β bonds, $J_{C\text{-}H}$=161.3–163.7 Hz), 93.62 (C-1 for α bond, $J_{C\text{-}H}$=174.8 Hz), 81.60, 78.53, 74.48, 26.43, 25.93, 20.34, 20.21, 20.21, 20.21, 20.00, 20.00. Anal.

calcd for $C_{135}H_{126}O_{49}$: C, 64.03; H, 5.01. Found: C, 64.51; H, 4.93.

Orthoester 17′;. To a stirred solution of **9** (3.6 g, 2.3 mmol) and **13** (2.5 g, 2.2 mmol) in CH_2Cl_2 (80 mL) was added TMSOTf (20 μL) at rt. After 20 min the mixture was neutralized with triethylamine and concentrated. The residue was purified on a silica gel column with 1.5:1 petroleum ether–EtOAc as the eluent to give the product **17′** (4.2 g, 76%). $[\alpha]_D$ +25.9 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.08, 169.65, 168.73, 168.50, 168.38, 121.77, 112.09, 104.62 (C-1 for glucofuranose α bond, $J_{C\text{-}H}$ = 182.4 Hz), 101.12, 100.59, 100.56, 98.00 (4 C-1 for β bonds, $J_{C\text{-}H}$ = 161.3–163.7 Hz), 96.32 (C-1 for α bond, $J_{C\text{-}H}$ = 174.8 Hz), 81.89, 78.75, 20.41, 20.22, 20.19, 20.11, 19.98. Anal. calcd for $C_{135}H_{126}O_{49}$: C, 64.03; H, 5.01. Found: C, 63.57; H, 4.87.

β-D-Glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-D-glucopyranose (18). Compound **17** (10 g, 3.95 mmol) was dissolved in 80% acetic acid solution (150 mL) and the mixture was heated under reflux for 5 h. The solution was concentrated, the residue was purified by chromatography with 1.5:1 petroleum ether–EtOAc as the eluent, and then the product was treated with a saturated solution of ammonia in CH_2Cl_2 (10 mL) and CH_3OH (120 mL) at rt. After 24 h, the reaction mixture was concentrated, and the residue was washed four times with CH_2Cl_2 to afford **18** as white solid (3.6 g, 92%). $[\alpha]_D$ −19.8 (*c* 0.1, H_2O); ^1H NMR (400 MHz, D_2O): δ 5.27 (d, 1H, *J* = 3.1 Hz), 4.74 (d, 1H, *J* = 6.8 Hz), 4.65 (d, 1H, *J* = 6.8 Hz), 4.64 (d, 1H, *J* = 6.8 Hz),4.44 (d, 1H, *J* = 6.8 Hz),4.43 (d, 1H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, D_2O): δ 102.69, 102.69, 102.6, 102.60, 102.60 (5 C-1, $J_{C\text{-}H}$ = 163.9 Hz), 98.92 (C-1, $J_{C\text{-}H}$ = 173.9 Hz), 85.34, 84.87, 82.9, 81.96. Anal. calcd for $C_{36}H_{62}O_{31}$: C, 43.64; H, 6.30. Found: C, 42.92; H, 6.89. ESMS for $C_{36}H_{62}O_{31}$ (990.86): 989.84 $[M\text{-}1]^+$.

Lauryl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-glucopyranoside (19). To a stirred solution of **9** (2.30 g, 1.47 mmol) and **16** (1.75 g, 1.35 mmol) in CH_2Cl_2 (60 mL) was added TMSOTf (30 μL) at rt. After 3 h, the mixture was neutralized with triethylamine and concentrated. The residue was purified on a silica gel column with 1.5:1 petroleum ether–EtOAc as the eluent to give **19** (3.1 g, 84%). $[\alpha]_D$ +48.5 (*c* 1.7, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.47, 170.10, 169.62, 169.51, 169.50, 169.38, 168.76, 101.40, 101.28, 101.13, 100.65, 100.40, 93.16, 31.88–21.24, 20.75, 20.69, 20.65, 20.58, 20.48, 20.42, 14.09. Anal. calcd for $C_{146}H_{148}O_{50}$: C, 64.88; H, 5.52. Found: C, 64.43; H, 5.58.

Lauryl β-D-Glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (20). Compound **19** (1.6 g, 0.59 mmol) was dissolved in a saturated solution of ammonia in CH_2Cl_2 (10 mL) and CH_3OH (100 mL) at rt. After 24 h, the reaction mixture was concentrated, and the residue was washed four times with CH_2Cl_2 to afford **20** as white solid (650 mg, 95%).$[\alpha]_D$ −13.6 (*c* 0.1, H_2O); ^1H NMR (400 MHz, D_2O): δ 5.33 (d, 1H, *J* = 3.2 Hz), 4.73, 4.50, 4.46, 4.41, 4.21 (5 d, 5H, *J* = 7.5 Hz); 4.20–3.36 (m, 38H), 1.67 (m, 2H), 1.45–1.21 (m, 18H), 0.92 (t, 3H); ^{13}C NMR (100 MHz, D_2O): δ 105.22, 105.06, 105.06, 104.95, 104.66, 101.23, 86.51, 85.55, 84.31. Anal. calcd for $C_{48}H_{86}O_{31}$: C, 49.74; H, 7.48. Found: C, 49.02; H, 7.89. ESMS for $C_{48}H_{86}O_{31}$ (1159.18): 1158.17 $[M\text{-}1]^+$.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-3-*O*-allyl-1,2-*O*-isopropylidene-α-D-glucofuranose (21). To a stirred solution of **2** (3.02 g, 4.1 mmol) and **4** (1.04 g, 4.0 mmol) in CH_2Cl_2 (50 mL) was added TMSOTf (20 μL) at room temperature. After 3 h, triethylamine was added to the solution to quench the reaction. The solution was concentrated, the residue was subjected to column chromatography with 3:1 petroleum ether–EtOAc as the eluent to give **21** (2.82 g, 84%) as a syrup: $[\alpha]_D$ +29.1° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.07–7.27 (m, 20H), 5.92 (t, 1H, *J* = 9.8 Hz), 5.88 (m, 1H), 5.84 (d, 1H, *J* = 3.0 Hz), 5.69 (t, 1H, *J* = 9.8 Hz), 5.54 (dd, 1H, *J* = 8.0, 9.6 Hz), 5.28 (dd, 1H), 5.17 (dd, 1H), 4.94 (d, 1H, *J* = 8.0 Hz), 4.67 (dd, 1H, *J* = 12.4, 3.2 Hz), 4.50 (d, 1H, *J* = 3.0 Hz), 4.48 (dd, 1H, *J* = 12.4, 3.2 Hz), 4.12–3.86 (m, 8H), 1.39, 1.28 (2 s, 6H). Anal. calcd for $C_{46}H_{46}O_{15}$: C, 65.86; H, 5.53. Found: C, 65.39; H, 5.41.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-glucofuranose (23). To a solution of **21** (4.8 g, 5.72 mmol) in pyridine (50 mL) was added BzCl (1.2 mL, 10 mmol) at rt. After 2 h, the mixture was poured to water and extracted with CH_2Cl_2. The organic phase was concentrated, and the resulting residue was treated with CH_3OH (100 mL) and $PdCl_2$ (15 mg) at rt for 4 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, and the filtrate was concentrated. The residue was passed a silica-gel column with 2:1 petroleum ether–EtOAc as the eluent to give **23** (4.0 g, 78%) as a syrup; $[\alpha]_D$ +34.3° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.89–7.25 (m, 25H), 5.91 (d, 1H, *J* = 3.0 Hz), 5.87 (t, 1H, *J* = 9.8 Hz), 5.68 (t, 1H, *J* = 9.8 Hz), 5.56 (dd, 1H, *J* = 8.1, 9.6 Hz), 5.31 (m, 1H), 5.05 (d, 1H, *J* = 8.1 Hz), 4.62 (dd, 1H, *J* = 12.1, 3.3 Hz), 4.55 (d, 1H, *J* = 3.0 Hz), 4.46 (dd, 1H, *J* = 12.4, 3.3 Hz), 4.36–3.99 (m, 5H), 1.50, 1.31 (2 s, 6H). Anal. calcd for $C_{50}H_{46}O_{16}$: C, 66.51; H, 5.13. Found: C, 66.11; H, 5.07.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,4-di-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl trichloroacetimidate (24). Compound **21** (12 g, 14.3 mmol) was added to 80% aqueous acetic acid solution (150 mL) and the mixture was heated under reflux for 5 h. The mixture was concentrated and the residue was acetylated with acetic anhydride (20 mL) in pyridine (20 mL) for 2 h at rt. The resultant trisaccharide was dissolved in a 1.5 N

solution of NH_3 in 3:1 THF-CH_3OH (100 mL), and the solution was kept at rt. After 3 h, the solution was concentrated, and the residue was dissolved in CH_2Cl_2 (80 mL). To the solution were added K_2CO_3 (3,4 g, 25 mmol) and CCl_3CN (2 mL, 20 mmol), and the mixture was stirred at rt for 24 h. Filtering the mixture, the filtration and washings were concentrated, and the residue was subjected to column chromatography to give **24** (11 g, 75%) as a syrup: $[\alpha]_D$ +42.4° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.35 (s, 1H), 8.04–7.26 (m, 20H), 6.32 (d, 1H, J=3.2 Hz), 5.86 (t, 1H, J=9.6 Hz), 5.75 (m, 1H), 5.64 (t, 1H, J=9.8 Hz), 5.48 (dd, 1H, J=8.0, 9.6 Hz), 5.16 (dd, 1H), 5.10 (dd, 1H), 4.96 (d, 1H, J=8.0 Hz), 4.86 (t, 1H, J=9.8 Hz), 4.84 (dd, 1H, J=10.4, 3.0 Hz), 4.61 (dd, 1H, J=10.4, 3.2 Hz), 4.49 (dd, 1H, J=9.6, 3.2 Hz), 4.17–3.90(m, 5H), 3.86 (t, 1H), 3.71 (dd, 1H), 2.04, 2.00 (2 s, 6H). Anal. calcd for $C_{49}H_{46}NO_{17}Cl_3$: C, 57.29; H, 4.51. Found: C, 57.71; H, 4.35.

Lauryl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,4-di-*O*-acetyl-3-*O*-allyl-β-D-glucopyranoside (25). To a solution of **24** (2.5 g, 2.43 mmol) and lauryl alcohol (744 mg, 4.0 mmol) in CH_2Cl_2 (50 mL) was added TMSOTf (30 μL) at rt. The reaction mixture was stirred for 3 h, at the end of which time TLC indicated that the reaction was complete. Then the mixture was neutralized with triethylamine and concentrated under reduced pressure to dryness. Purification by column chromatography (3:1 petroleum ether–EtOAc) gave **25** as a syrup (2.35 g, 92%):$[\alpha]_D$ +33.7′ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.04–7.27 (m, 20H), 5.88 (t, 1H, J=9.7 Hz), 5.71 (m, 1H), 5.66 (t, 1H, J=9.6 Hz), 5.51 (dd, 1H, J=8.0, 9.6 Hz), 5.16 (dd, 1H), 5.10 (dd, 1H), 4.95 (d, 1H, J=8.0 Hz), 4.85 (t, 1H, J=9.8 Hz), 4.78 (t, 1H, J=9.8 Hz), 4.63 (dd, 1H, J=10.4, 2.0 Hz), 4.47 (dd, 1H, J=10.4, 3.2 Hz), 4.19 (d, 1H, J=9.4 Hz), 4.15 (m, 1H), 4.01 (d, 2H), 3.88 (d, 1H), 3.65 (t, 1H), 3.60–3.40 (m, 3H), 3.05 (m, 1H), 2.04, 2.00 (2 s, 6H), 1.33–1.12 (m, 20H), 0.88 (t, 3H). Anal. calcd for $C_{59}H_{70}O_{17}$: C, 67.41; H, 6.71. Found: C, 67.02; H, 6.59.

Lauryl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,4-di-*O*-acetyl-β-D-glucopyranoside (26). To a solution of **25** (3.4 g, 3.23 mmol) in CH_3OH (50 mL) was added $PdCl_2$ (20 mg), and the mixture was stirred for 4 h at rt, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, the filtrate was concentrated, and the residue was passed a silica-gel column with 2:1 petroleum ether–EtOAc as the eluent to give **26** as a syrup (2.71 g, 83%): $[\alpha]_D$ +14.9° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.05–7.25 (m, 20H), 5.89 (t, 1H, J=9.6 Hz), 5.68 (t, 1H, J=9.6 Hz), 5.52 (dd, 1H, J=7.8, 9.6 Hz), 4.95 (d, 1H, J=7.8 Hz), 4.75–4.67 (m, 3H), 4.47 (dd, 1H, J=4.9, 12.1 Hz), 4.24 (d, 1H, J=7.8 Hz), 4.15 (m, 1H), 3.92 (dd, 1H), 3.72–3.50 (m, 4H), 3.12 (m, 1H), 2.07, 2.02 (2 s, 6H), 1.38–1.11 (m, 20H), 0.88 (t, 3H, J=6.6 Hz). Anal. calcd for $C_{56}H_{66}O_{17}$: C, 66.52; H, 6.58. Found: C, 66.05; H, 6.74.

4-Methoxyphenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-glucopyransyl-(1→3)-4,6-*O*-benzylidene-β-D-glucopyranoside (28). To a stirred solution of **9** (3.6 g, 2.3 mmol) and **27**[13] (865 mg, 2.3 mmol) in CH_2Cl_2 (30 mL) was added TMSOTf (30 μL) at room temperature. After 3 h, triethylamine was added to the solution to quench the reaction. The solution was concentrated, the residue was subjected to column chromatography with 1.5:1 petroleum ether–EtOAc as the eluent to give **28** (3.15 g, 77%): $[\alpha]_D$ −10.3° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.03–7.22 (m, 45H), 7.10, 6.83 (2 d, 4H, J=9.1 Hz), 5.89 (t, 1H, J=9.7 Hz), 5.73 (t, 1H, J=9.4 Hz), 5.68–5.62 (m, 2H), 5.43–5.36 (m, 2H), 5.38 (s, 1H), 5.00 (t, 1H, J=8.7 Hz), 4.89 (d, 1H, J=7.6 Hz), 4.87 (dd, 1H, J=7.8 Hz), 4.74 (d, 1H, J=7.9 Hz), 4.60 (dd, 1H), 4.45 (m, 2H), 4.25–4.05 (m, 3H), 3.76 (s, 3H), 1.94, 1.91 (2 s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.88, 168.96, 166.15-164.80, 155.60, 151.26, 117.82, 114.68, 101.70, 101.10, 100.70, 100.38. Anal. calcd for $C_{98}H_{88}O_{32}$: C, 66.21; H, 4.99. Found: C, 66.64; H, 4.89.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-]2,4-di-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (29). A solution of **28** (5 g, 2.81 mmol) in 90% acetic acid (80 mL) was kept at 40 °C for 24 h and then concentrated to a residue under reduced pressure. The residue was treated with pyridine (10 mL) and Ac_2O (10 mL) at rt for 2 h. This mixture was added to water and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, and concentrated, the residue was dissolved in 4:1 CH_3CN–H_2O (40 mL). To this solution was added $(NH_4)_2Ce(NO_3)_6$ (2.72 g, 5.0 mmol), and the mixture was stirred for 30 min at rt, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was extracted with CH_2Cl_2, and washed with satd aq $NaHCO_3$. The organic layer was concentrated, the residue was dissolved in dichloromethane. To the solution were added K_2CO_3 (1.4 g, 10 mmol) and CCl_3CN (0.5 mL, 5 mmol), and the mixture was stirred at rt for 24 h. Filtering the mixture, the filtration and washings were concentrated, and the residue was subjected to column chromatography (1.5:1 petroleum ether–EtOAc) to give **29** (3.7 g, 71%): $[\alpha]_D$ +4.0° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.65 (s, 1H), 8.15–7.24 (m, 40H), 6.38 (d, 1H, J=3.5 Hz), 5.88 (t, 1H, J=9.4 Hz), 5.87 (t, 1H, J=9.4 Hz), 5.66 (t, 1H, J=9.4 Hz), 5.64 (t, 1H, J=9.3 Hz), 5.50 (dd, 1H, J=7.8, 9.0 Hz), 5.37 (dd, 1H, J=7.8, 9.0 Hz), 4.92–4.83 (m, 4H), 4.74–4.63 (m, 3H), 4.60–4.45 (m, 3H), 4.40 (d, 1H, J=8.1 Hz), 4.16–3.56 (m, 10H), 2.08, 2.02, 1.86, 1.81, 1.74 (5 s, 15H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.77, 169.73, 169.71, 169.01, 168.09, 160.99, 101.32, 100.79, 100.66, 93.23, 20.72, 20.68, 20.60, 20.50, 20.32. Anal. calcd for $C_{92}H_{84}NO_{34}Cl_3$: C, 59.60; H, 4.57. Found: C, 59.09; H, 4.63.

Lauryl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-

benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-O-acetyl-β-D-glucopyranoside (30) and Lauryl 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-O-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-O-acetyl-α-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-O-acetyl-β-D-glucopyranoside (31). To a stirred solution of **29** (3.6 g, 1.94 mmol) and **26** (1.82 g, 1.8 mmol) in CH_2Cl_2 (30 mL) was added TMSOTf (20 μL) at room temperature. After 3 h, triethylamine was added to the solution to quench the reaction. The solution was concentrated, the residue was subjected to column chromatography with 1:1 petroleum ether–EtOAc as the eluent to give **30** (1.85 mg, 38%) and **31** (838 mg, 16%): For **30**: $[\alpha]_D$ +38° (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.35, 169.52, 169.38, 168.82, 168.63, 168.63, 168.28, 101.18, 100.18, 100.28, 100.28, 100.19, 100.19 (6 C-1, $J_{C\text{-}H}$ = 163.2-163.3 Hz), 78.45, 77.96, 77.94, 20.71, 20.65, 20.55, 20.47, 20.38, 20.30, 20.25. Anal. calcd for $C_{146}H_{148}O_{50}$: C, 64.88; H, 5.52. Found: C, 64.40; H, 5.65; For **31**: $[\alpha]_D$ +8.5° (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.52, 170.27, 169.67, 169.29, 169.06, 168.87, 167.88, 101.15, 101.08, 100.84, 100.54, 100.54, 95.19, 20.91, 20.80, 20.79, 20.65, 20.48, 40.30, 20.30. Anal. calcd for $C_{146}H_{148}O_{50}$: C, 64.88; H, 5.52. Found: C, 64.56; H, 5.47.

Orthoester 30′. To a stirred solution of **29** (1.30 g, 0.7 mmol) and **26** (0.71 g, 0.7 mmol) in CH_2Cl_2 (20 mL) was added TMSOTf (10 μL) at room temperature. After 20 min, triethylamine was added to the solution to quench the reaction. The solution was concentrated, the residue was subjected to column chromatography with 1.5:1 petroleum ether–EtOAc as the eluent to give **30′** (1.32 g, 71%): $[\alpha]_D$ +17.6° (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.53, 169.57, 169.46, 169.46, 169.08, 167.92, 121.90, 101.20, 101.04, 100.49, 100.38, 100.28, 96.60. Anal. calcd for $C_{146}H_{148}O_{50}$: C, 64.88; H, 5.52. Found: C, 65.22; H, 5.67.

Lauryl β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl(1→6)]-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (32). Compound **30** (1.2 g, 0.44 mmol) was dissolved in a saturated solution of NH_3 in CH_2Cl_2 (5 mL) and CH_3OH (50 mL) at rt. After 24 h, the reaction mixture was concentrated, and the residue was washed four times with CH_2Cl_2 to afford **32** as white solid (470 mg, 92%): $[\alpha]_D$ −8.4° (*c* 1.0, H_2O); ^1H NMR (400 MHz, D_2O): δ 4.39–3.98 (m, 6H), 3.85–3.09 (m, 38H), 1.51 (m, 2H), 1.22–1.12 (m, 18H,), 0.76 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 105.52, 105.33, 105.23, 105.18, 105.14, 104.94 (6 C-1), 88.16, 87.42, 86.81. Anal. calcd for $C_{48}H_{86}O_{31}$: C, 49.74; H, 7.48. Found: C, 49.07; H, 7.76. ESMS for $C_{48}H_{86}O_{31}$ (1159.18): 1158.16 $[M-1]^+$.

Lauryl β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl (1→6)]-β-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (33). Compound **31** (470 mg, 0.17 mmol) was dissolved in a solution of CH_2Cl_2 (5 mL) and CH_3OH (50 mL) saturated with NH_3 at rt. After 24 h, the reaction mixture was concentrated, and the residue was washed four times with CH_2Cl_2 to afford **33** as white solid (183 mg, 93%). $[\alpha]_D$ −12.1° (*c* 1.0, H_2O); ^{13}C NMR (100 MHz, $CDCl_3$): δ 102.70, 102.68, 102.68, 102.65, 102.65 (5 C-1, $J_{C\text{-}H}$ = 164.2-164.9 Hz), 99.15 (C-1, $J_{C\text{-}H}$ = 175.44 Hz), 84.24, 83.47, 82.23. Anal. calcd for $C_{48}H_{86}O_{31}$: C, 49.74; H, 7.48. Found: C, 49.17; H, 7.89. ESMS for $C_{48}H_{86}O_{31}$ (1159.18): 1158.17 $[M-1]^+$.

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Pure α-linked products can be obtained in high yields in glycosylation with glucosyl trichloroacetimidate donors with a C2 ester capable of neighboring group participation

Ying Zeng, Jun Ning* and Fanzuo Kong*

Research Center for Eco-Environmental Sciences, Academia Sinica, PO Box 2871, Beijing 100085, PR China

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Abstract—Predominant or even pure (1→3)-α-linked products can be generated in glycosylation with glucosyl trichloroacetimidate donors with a C2 ester capable of neighboring group participation. Benzoylation of either the donors or acceptors gave more β-linkage, while 4,6-*O*-benzylidenation of the acceptor gave exclusive β-glucosylation. 3-*O*-Glycosylation of the donor and the presence of a (1→3)-β-linkage in the oligosaccharide acceptor gave sole α-glucosylation. © 2002 Elsevier Science Ltd. All rights reserved.

Neighboring group participation has been frequently used in organic synthesis. In carbohydrate chemistry, generally, it is believed that glycosyl donors possessing an acyloxyl group with a participating function at C-2 exclusively give the corresponding 1,2-*trans* glycoside with quite high stereoselectivity in glycosylation reactions. Therefore, the most widely used approach for achieving stereochemical control in the formation of β-glucosidic linkages involves the use of a C2 ester capable of neighboring group participation.[1] In this type of chemistry, *ortho* esters are frequent intermediates[2] or undesired byproducts[3] owing to trapping of the intermediate bridging cation by the nucleophile as opposed to attack at the anomeric carbon. Whitfield has published calculations that address the mechanism of neighboring group participation that support the notion of a bridging cation as an intermediate.[4] The orthoesters formed during glycosylation, in the absence of a buffer, subsequently rearrange to the corresponding 1,2-*trans* glycosidic products under the action of protic or Lewis acids.[3b–d,5] This transformation has recently been put into good use.[6]

As part of our program to develop an immune stimulant, we are dealing with synthesis of (1→3)-β-D-glucooligosaccharides. These oligosaccharides, which are the fragments of the natural (1→3)-β-D-glucan homopolysaccharides isolated from the inner cell wall of *Saccharomyces cerevisiae*,[7] stimulate immunity and belong to the class of drugs known as biological response modifiers (BRMs). For an investigation of structure–immunity relationships, we needed a series of (1→3)-β-D-glucooligosaccharides. Thus, we prepared 3-*O*-allyl-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-D-glucopyranosyl trichloroacetimidate (**1**) as the donor and lauryl 2,4,6-tri-*O*-benzoyl-β-D-glucopyranoside (**2**) as the acceptor (entry 1, Fig. 1). We expected that the coupling of **1** with **2** in the presence of TMSOTf[8] would give a β-linked trisaccharide. However, to our surprise, the donor and acceptor were linked with a pure α-bond. Similar anomalous stereoselectivity was observed by Hashimoto and Izumi when they carried out the glycosylation with peracetylated 5-thio-D-arabinopyranosyl and 5-thio-L-fucopyranosyl trichloroacetimidates.[9] To explore the stereoselectivity–structure relationship, a variety of donors and acceptors were used for coupling. Fig. 1 shows that the stereoselectivity of the glycosylation was dependent on the structures of both the acceptor and the donor.

It was found, see Fig. 1, that 4,6-*O*-benzylidenation of the acceptor favored β-linkage formation (entry 2), while benzoylation of the donor and acceptor instead of acetylation tended to give more β-glycosylation as indicated from entry 3 versus entries 4 and 5. The former entry gave sole β-linkage, while the latter two entries gave α- and β-linked mixtures (α:β = 1:2 and 1:1, respectively). However, 3-*O*-allylation of the donor substan-

* Corresponding authors.

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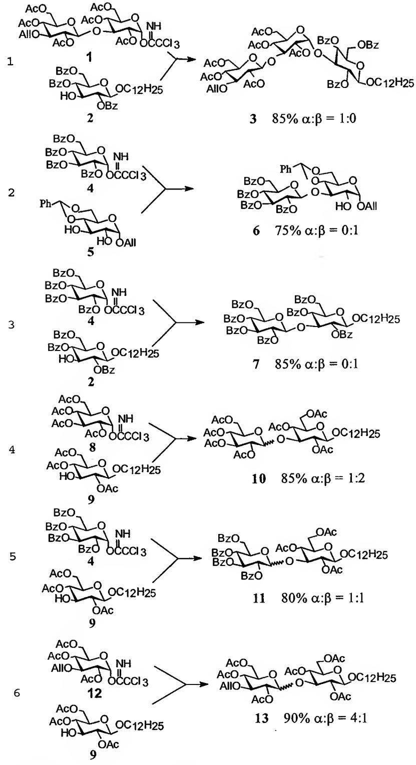
tially changed the stereoselectivity yielding predominant α-linkage (α:β 4:1) as indicated in entry 6. Moreover, 3-*O*-glycosylation completely changed the stereoselectivity to sole α-linkage as shown in entries 1 and 7. A (1→3)-β-linked disaccharide as the acceptor also gave α-glycosylation (entry 8). Similarly, condensation of a (1→3)-β-linked disaccharide donor **18** with a trisaccharide acceptor **19** having a (1→3)-β-linkage at the non-reducing end yielded sole α-linked pentasaccharide **21** (entry 9).

The stereoselectivity of the glycosylation was readily determined by ^{1}H and ^{13}C NMR spectroscopy. For di- and trisaccharides, ^{1}H NMR usually gave clear



1: **1**, **2** → **3** 85% α:β = 1:0

2: **4**, **5** → **6** 75% α:β = 0:1

3: **4**, **2** → **7** 85% α:β = 0:1

4: **8**, **9** → **10** 85% α:β = 1:2

5: **4**, **9** → **11** 80% α:β = 1:1

6: **12**, **9** → **13** 90% α:β = 4:1

Figure 1. Coupling results with different donors and acceptors.

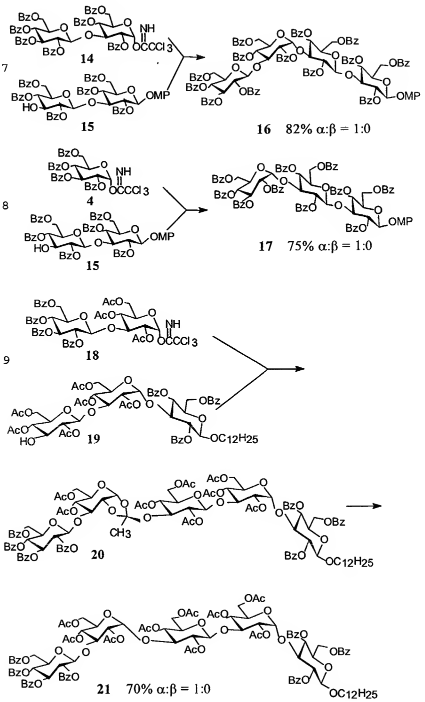


Fig. 1. (*Continued*)

identification since the signals in the 4–6 ppm region were well resolved and H-1α, H-1β showed coupling constants of ~3 and ~8 Hz, respectively. For higher oligosaccharides, ^{13}C NMR spectra were also recorded giving the J_{C1-H1} at δ 173–179 Hz for an α-linkage, and at 159–166 Hz for a β-linkage.[10]

In the coupling reactions, it was found that orthoesters were formed at the initial stage, and they rearranged quickly to the final products. This was readily monitored by TLC since the orthoester intermediates and the final products gave different R_f values. Meanwhile, the orthoesters could be isolated, and they were easily transformed to the final products by treatment with catalytic TMSOTf. In entry 9, the orthoester **20** was isolated and identified by ^{1}H and ^{13}C NMR, and it was then converted to the pentasaccharide **21** by treatment with catalytic TMSOTf.

Following the literature precedence[4,11] we hypothesize herein a possible mechanism as shown in Fig. 2. Activation of the glycosyl donor **I** with a promoter may lead to irreversible formation of a glycosyl oxocarbenium

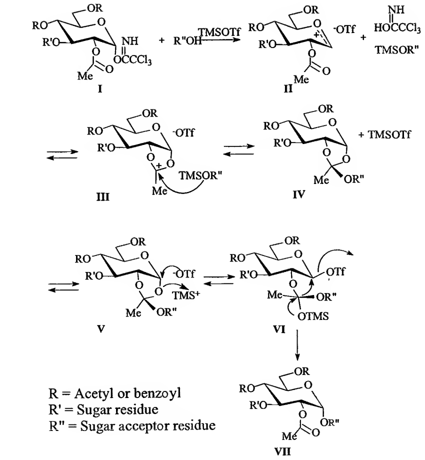


Figure 2. A proposed mechanism for α-linkage formation from an orthoester.

ion **II**. Previous work and the calculations of Whitfield pointed out that the bridging dioxolenium ion **III** is an intermediate, and nucleophiles can react with this ion giving orthoester **IV**. If the nucleophile attacks the C-1 rear side of **III** or the OR′ in **IV** rearranges to reach the C-1 rear side, the normal β-linkage is obtained. However, if TMSOTf promoted C-1–O bond breaking occurs in **V**, an intermediate **VI** will form, and its subsequent rearrangement will give the 1,2-*cis* linked glycoside **VII**.

In summary, the findings described above indicated that the (1→3)-α-linked glucosidic bond can be constructed using fully acylated glucosyl trichloroacetimidates as the donors. This can be used in the synthesis of some biologically active glucans containing (1→3)-α-linkages.

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8. Typical glycosylation conditions: the glycosyl donor (0.5 mmol) and acceptor (0.5 mmol) were dried together under high vacuum for 2 h, then dissolved in anhydrous CH_2Cl_2 (30 mL). TMSOTf (15 μL, 0.05 equiv.) was added dropwise at rt. The reaction mixture was stirred for 3 h after which the mixture was neutralized with Et_3N and concentrated to dryness under reduced pressure. Purification by column chromatography afforded the final product.

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10. All new compounds involved in this study were identified by optical rotations, ^1H or ^{13}C NMR spectroscopy, and elemental analyses. Selected spectral data: **3** ^1H NMR ($CDCl_3$): δ 7.99–7.35 (15H), 5.65 (m, 1H, =C*H*-), 5.57 (t, 1H, J=9.1 Hz), 5.36 (t, 1H, J=9.1 Hz), 5.11 (d, 1H, J=2.0 Hz, α-H-1), 5.10 (m, 2H), 4.77 (t, 2H), 4.66 (d, 1H, J=8 Hz, β-H-1), 4.61 (dd, 1H, J=3.6, 10.4 Hz), 4.50 (m, 1H), 4.42 (d, 1H, J=8 Hz, β-H-1), 4.41 (m, 1H), 4.31 (t, 1H, J=9.2 Hz), 4.25 (m, 1H), 4.11 (m, 1H), 3.98–3.93 (m, 4H), 3.84 (m, 1H), 3.62 (m, 1H), 3.46–3.39 (m, 2H), 2.12 (s, 3H), 2.09 (s, 3H), 1.97 (s, 3H), 1.94 (s, 3H), 1.87 (s, 3H), 1.75 (s, 3H), 1.53–0.90 (m, 21H), 0.86 (t, 3H). ^{13}C NMR ($CDCl_3$): δ 170.15, 169.91, 169.00, 168.66, 168.17, 168.08, 165.53, 164.57, 164.40, 133.78, 116.20, 100.63 (J_{C1-H1}=159 Hz, β-C-1), 100.28 (J_{C1-H1}=162 Hz, β-C-1), 95.63 (J_{C1-H1}=173 Hz, α-C-1), 79.64, 75.91, 74.58, 71.97, 71.81, 71.59, 71.59, 71.41, 69.68, 69.07, 67.48, 66.89, 62.86, 61.83, 60.69, 31.36, 29.04, 28.95, 28.77, 28.69, 25.29, 22.11, 20.09, 19.97, 19.67, 13.50. **6** (2-OH was benzoylated with BzCl–pyridine). ^1H NMR ($CDCl_3$): δ 8.13–7.21 (25H), 6.92–6.90 (m, 4H), 5.89 (t, 1H, J=9.6 Hz), 5.65 (m, 1H), 5.60 (t, 1H, J=9.2 Hz), 5.53 (t, 1H, J=8 Hz), 5.18–5.12 (m, 3H), 5.07 (d, 1H, J=3.2 Hz, α-H-1), 5.04 (d, 1H, 8 Hz, β-H-1), 4.93 (dd, 1H, J=4.1, 10.0 Hz), 4.80 (m, 1H), 4.43 (m, 1H), 4.29 (m, 1H), 4.18–4.08 (m, 2H), 3.93–3.73 (m, 5H). **7**. ^1H NMR ($CDCl_3$): δ 5.01 (d, 1H, J=7.6 Hz, β-H-1), 4.60 (d, 1H, J=8 Hz, β-H-1). **10** (the α and β anomers could not be separated) α anomer ^1H NMR ($CDCl_3$): δ 5.27 (d, 1H, J=3.2 Hz, α-H-1), 4.57 (d, 1H, J=8 Hz, β-H-1); β anomer: 4.57 (d, 1H, J=8 Hz, β-H-1), 4.33 (d, 1H, J=7.6 Hz, β-H-1). ^{13}C NMR ($CDCl_3$) of β anomer: 100.99 (β-C-1), 100.85 (β-C-1); α anomer: δ 100.99 (β-C-1), 96.15 (α-C-1). **11** β anomer ^1H NMR ($CDCl_3$): δ 4.97 (d, 1H, J=7.6 Hz, β-H-1), 4.29 (d, 1H, J=7.6 Hz, β-H-1). ^{13}C NMR ($CDCl_3$): δ 100.74 (β-C-1), 100.41 (β-C-1). α anomer ^1H NMR ($CDCl_3$): δ 5.52 (d, 1H, J=3.6 Hz, α-H-1), 5.02 (d, 1H, J=8.0 Hz, β-H-1); ^{13}C NMR($CDCl_3$): δ 100.78 (β-C-1), 95.24 (α-C-1). **13** β anomer ^1H NMR ($CDCl_3$): δ 4.59 (d, 1H, J=8 Hz, β-H-1), 4.34 (d, 1H, J=8.0 Hz, β-H-1); ^{13}C NMR ($CDCl_3$): δ 101.27 (β-C-1), 101.11 (β-C-1); α isomer ^1H NMR ($CDCl_3$): δ 5.20 (d, 1H, J=3.6 Hz, α-H-1), 4.66 (d, 1H, J=8.0 Hz, β-H-1); ^{13}C NMR ($CDCl_3$): δ 100.98 (β-C-1), 95.94 (α-C-1). **16**. ^1H NMR ($CDCl_3$): δ 5.38 (d, 1H, J=3.6 Hz, α-H-1), 5.02 (d, 1H, J=8 Hz, β-H-1), 4.97 (d, 1H, J=6.8 Hz, β-H-1), 4.45 (d, 1H, J=7.6 Hz, β-H-1); ^{13}C NMR ($CDCl_3$): δ 101.00 (β-C-1), 99.98 (β-C-1), 99.88 (β-C-1), 95.67 (α-C-1). **17**. ^1H NMR ($CDCl_3$): δ 5.53 (d, 1H, J=4 Hz, α-H-1), 5.13 (d, 1H, J=8 Hz, β-H-1), 5.09 (d, 1H, J=7.6 Hz, β-H-1). ^{13}C NMR ($CDCl_3$): δ 101.01 (β-C-1), 100.95 (β-C-1), 96.21 (α-C-1). **20**. ^1H NMR ($CDCl_3$): δ 5.51 (d, 1H, J=4.0 Hz, α-H-1), 5.14 (d, 1H, J=3.6 Hz, α-H-1), 4.68 (d, 1H, J=8.0 Hz, β-H-1); ^{13}C NMR ($CDCl_3$): δ 101.60 (J_{C1-H1}=167 Hz, β-C-1), 100.67 (J_{C1-H1}=160 Hz, β-C-1), 99.87 (J_{C1-H1}=160 Hz, β-C-1), 96.52 (J_{C1-H1}=177 Hz, α-C-1), 95.71 (J_{C1-H1}=177 Hz, α-C-1). **21**. ^1H NMR ($CDCl_3$): δ 5.14 (d, 1H, J=3.6 Hz, α-H-1), 5.11 (d, 1H, J=3.2 Hz, α-H-1), 4.95 (d, 1H, J=8.0 Hz, β-H-1), 4.85 (d, 1H, J=8.4 Hz, β-H-1), 4.43 (d, 1H, J=8.0 Hz, β-H-1); ^{13}C NMR ($CDCl_3$): δ 100.78 (J_{C1-H1}=165 Hz, β-C-1), 100.68 (J_{C1-H1}=163 Hz, β-C-1), 99.91 (J_{C1-H1}=166 Hz, β-C-1), 95.67 (J_{C1-H1}=173 Hz, α-C-1), 94.82 (J_{C1-H1}=179 Hz, α-C-1).

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ORIGINAL RESEARCH

An Evaluation of the Immunological Activities of Commercially Available β1, 3-Glucans

Vaclav Vetvicka, PhD*, Jana Vetvickova, MS
University of Louisville, Department of Pathology, Louisville, Kentucky

ABSTRACT

Introduction

β1,3-glucan's role as a biologically active immunomodulator has been well documented for over 40 years. Interest in the immunomodulatory properties of polysaccharides was initially raised after experiments showing that a crude yeast cell preparation stimulated macrophages via activation of the complement system.[1] Further work identified the immunomodulatory active component as β1,3-glucan.[2] Numerous studies (currently more than 1,600 publications) have subsequently shown that β1,3-glucans, either particulate or soluble, exhibit immunostimulating properties, including antibacterial and anti-tumor activities.[3,4]

Despite extensive investigations, no consensus on the source, size and other biochemical or physicochemical properties of β1,3-glucan has been achieved. In addition, numerous concentrations and routes of administration have been tested – including oral, intraperitoneal, subcutaneous and intravenous applications.

* *Correspondence:*
Vaclav Vetvicka, PhD
University of Louisville
511 South Floyd
Louisville, Kentucky 40202
Phone: 502-852-1612 Fax: 502-852-1177
E-mail: vaclav.vetvicka@louisville.edu

This fact, together with the fact that there are probably more than a hundred different samples on the US market alone, leads to confusion about the quality, biological effects, and overall efficiency of glucan. Therefore, we decided to compare the basic immunological activities of a group of glucans. The list of products chosen came from those heavily advertised, commonly available and easily obtained in the US, Europe, Southeast Asia and Japan. In order to be certain that we are measuring the effects of glucan only, we picked the commercial samples with glucan (either from one source or a mixture of different glucans) as the only active ingredient.

The collection of tested biological reactions (phagocytosis, surface markers on splenocytes, cytokine synthesis, and stimulation of antibody response) represents both the humoral and cellular branches of the immune reaction, thus offering insight as to the immunological activities of studied glucans.

MATERIAL AND METHODS

Animals

Female, 6–to 10–week–old BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal work was done according to the University of Louisville IACUC protocol. Animals were sacrificed by CO_2 asphyxiation.

Materials

RPMI 1640 medium, sodium citrate, dextran, Ficoll-Hypaque, antibiotics, sodium azide, bovine serum albumin (BSA), Wright stain, Limulus lysate test E-TOXATE,

Freund's adjuvant and Concanavalin A were obtained from Sigma Chemical Co. (St. Louis, MO). Fetal calf serum (FCS) came from Hyclone Laboratories (Logan, UT).

β1,3-glucans

The glucans used in this study were purchased from the following companies: Now BETA glucan from Now Foods (Bloomingdale, IL), IMMUTOL from Biotec (Tromso, Norway), Immune Builder and Maitake Gold 404 from Mushroom Science (Eugene, OR), Glucan #300 from Transfer Point (Columbia, SC), Glucagel T from GraceLinc (Christchurch, New Zealand), and Senseiro from Sundory (Tokyo, Japan).

Antibodies

For fluorescence staining, the following antibodies have been employed: anti-mouse CD4, CD8 and CD19, conjugated with FITC were purchased from Biosource (Camarillo, CA).

Flow cytometry

Cells were stained with monoclonal antibodies on ice in 12x75-mm glass tubes using standard techniques. Pellets of $5x10^5$ cells were incubated with 10 μl of FITC-labeled antibodies (1 to 20 μg/ml in PBS) for 30 minutes on ice. After washing with cold PBS, the cells were re-suspended in PBS containing 1% BSA and 10 mM sodium azide. Flow cytometry was performed with a FACScan (Becton Dickinson, San Jose, CA) flow cytometer and the data from over 10,000 cells/samples were analyzed.

Phagocytosis

The technique employing phagocytosis of synthetic polymeric microspheres was described earlier.[5,6] Briefly: peritoneal cells were incubated with 0.05 ml of 2-hydroxyethyl methacrylate particles (HEMA; $5x10^8$/ml). The test tubes were incubated at 37° C for 60 min. with intermittent shaking. Smears were stained with Wright stain. The cells with three or more HEMA particles were considered positive. The same smears were also used for evaluation of cell types.

Evaluation of IL-2 production

Purified spleen cells ($2x10^6$/ml in RPMI 1640 medium with 5% FCS) were added into wells of a 24-well tissue culture plate. After addition of 1 mg of Concanavalin A into positive-control wells, cells were incubated for 72 hrs. in a humidified incubator (37°C, 5% CO_2). At the endpoint of incubation, supernatants were collected, filtered through 0.45 mm filters and tested for the presence of IL-2.[7] Levels of the IL-2 were measured using a Quantikine mouse IL-2 kit (R&D Systems, Minneapolis, MN).

RESULTS

The number of individual glucans is almost as great as the number of sources used for their isolation. The rationale for this combination of glucan samples was not only their commercial availability and success, but most importantly, we tried to include both soluble and insoluble glucans, and also glucans from different sources, including yeast, mushrooms and cereals (Table 1).

Glucagel barley β-glucan is a mixed link (13, 14)-ß-D-glucose polymer, in which cellotriosyl and cellotetraosyl residues occur in a ratio of ~3:1. The natural purification process yields a reduced molecular weight ß-glucan (typically ~130 kDa) that is more readily hydrated than other conventionally purified β-glucans. The typical carbohydrate content is 85–90%.

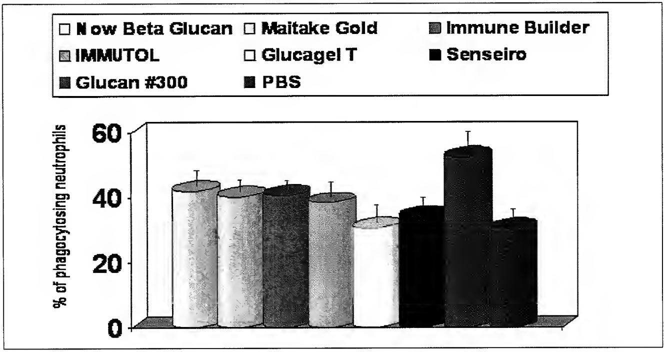
Senseiro is a soluble, high molecular weight glucan isolated from *Agaricus blazei,* consisting of approximately 63% carbohydrate. Glucan #300 is a proprietary (13, 16)-ß-D-glucan purified from *Saccharomyces cerevisiae* by Biothera for Transfer Point and even when corresponding to the glucan sold under WGP name, has much higher purity (app. over 96%).

β-glucans are generally considered to be potent stimulators of cellular immunity, with macrophages and neutrophils being the most important targets. Not surprisingly, we started our evaluation of glucan activities by phagocytosis. We used the synthetic polymeric microspheres, HEMA, since their use, dose and timing are already well established in glucan studies.[7-9] Results summarized in Figure 1 show significant effects of glucan samples on encapsulation of synthetic particles by peripheral blood neutrophils. The significant stimulation of phagocytic activity was found with five glucans – Now Beta Glucan, Maitake Gold, Immune Builder, IMMUTOL and Glucan #300. The other samples, with the exception of Glucagel T, also stimulated the phagocytosis, but at a much lower level and the results were not significant.

Next, we compared the effects of tested glucans on the expression of several membrane markers on splenocytes. Twenty-four hours after an ip. injection of 100 μg of individual glucan, spleen cells were isolated and the surface expression of CD4, CD8 and CD19 was evaluated by flow cytometery. The results summarized in Figure 2 demonstrated that only three glucans –Now Beta Glucan, Maitake Gold, and Glucan #300–significantly increased the migration of CD4- and CD8-positive T lymphocytes; none of the glucans had any significant effect on changes in the presence of CD19-positive B lymphocytes.

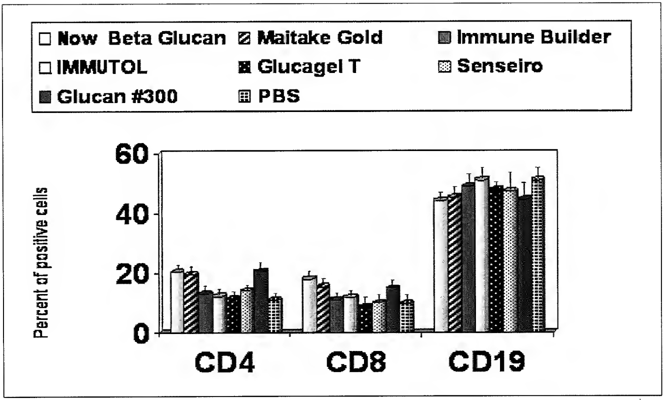
Evidence of the immunomodulating activity was also demonstrated through effects on the production of IL-2 by spleen cells (Figure 3). The production of IL-2 was measured after a 72 hr. in vitro incubation of spleen cells isolated from control and glucan-treated mice. Again, treatment of mice with Now Beta Glucan, Maitake Gold, and Glucan #300 showed the highest stimulation of IL-2 production. Immune Builder and IMMUTOL showed medium

Figure 1.



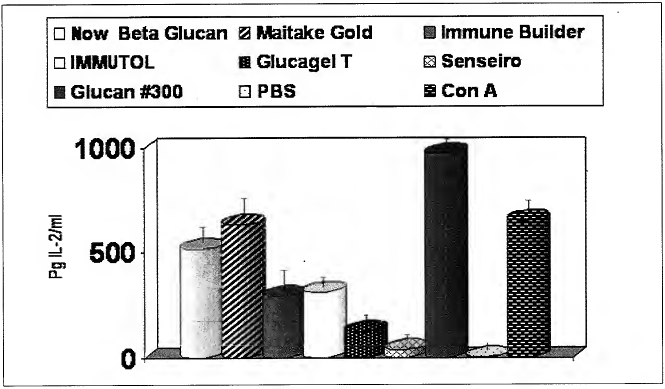
Effect of an ip. administration of 100 μg of different glucan samples on phagocytosis by peripheral blood granulocytes. Each value represents the mean ± SD. *Represents significant differences between control (PBS) and glucan samples at P ≤0.05 level.

Figure 2.



Effect of ip. injection of 100 μg of tested glucans on the expression of CD4, CD8 and CD19 markers by spleen cells. The cells from three donors at each time interval were examined and the results given represent the means ± SD. *Represents significant differences between control (PBS) and samples at P ≤ 0.05 level.

Figure 3.



Effects of glucans on Con A-stimulated secretion of IL-2 by spleen cells. *Represents significant differences between control (PBS) and samples at $P \leq 0.05$ level.

level stimulation. As the secretion of IL-2 by non-stimulated splenocytes (PBS group) is almost zero, even low stimulation by Glucagel T was significant. Another way to compare the effect on IL-2 formation and/or secretion is to compare it to the Con A stimulation. In this case, only Glucan #300 showed higher effects than Con A, whereas Now Beta Glucan and Maitake Gold were comparable, and the rest of the glucans showed much smaller effects.

We then focused on the use of glucan as an adjuvant. As an experimental model, we used immunization with ovalbumin. Glucans were applied together with two intraperitoneal doses of antigen; a commonly used Freund's adjuvant was used as additional positive control. The results (Figure 4) showed that only Immune Builder and Senseiro glucans had no effects on antibody response. All other glucans significantly supported the formation of specific antibodies. Glucans with the highest stimulation were Glucagel T and Glucan #300. It must be noted, however, that none of the glucans potentiated the humoral immunity to the level of Freund's adjuvant.

Table 2 summarizes the activities of individual glucans in all tested functions. Clearly, the most active samples were Glucan #300, followed by Now Beta Glucan and Maitake Gold 404. Senseiro glucan was almost without measurable activity.

DISCUSSION

Despite the extensive amount of scientific reports about glucans and their biological activities, most of the studies are focused on the description of chemical and/or biological properties of one particular glucan. Numerous types of glucans have been isolated from almost every species of yeast and fungi. For a long time, attention was focused mainly on glucans isolated from yeast and mushrooms. Recently, the existence of a highly purified linear β1,3-glucan named Phycarine, and subsequent study showing that Phycarine induced a broad range of defense reactions in tobacco cells,[10] brought new attention to seaweed-derived glucans.[11-13] More studies revealed that Phycarine significantly stimulated phagocytosis, synthesis and release of IL-1, IL-6 and TNF-α, and NK cell-mediated killing of tumor cells both in vitro and in vivo.[8] Similarly, recent clinical trials demonstrated the high activity of glucan isolated from barley.[14] It is clear, therefore, that the biological activities of glucans might be related more to the purity and biochemical/physiochemical characteristics than to the source.

Comprehensive reviews comparing several glucans are rare. However, in one of those studies, Yadomae reviewed how the structural properties of glucans affected biological activities and found that branched or linear 1,4–glucans

Table 1.

Glucan used in this study			
Name	**Source**	**Manufacturer/Distributor**	**Solubility**
β-1,3/1,6-D-glucan	*Saccharomyces cerevisiae* *Grifola frondosa*	Now Foods	No
MaitakeGold 404	*Grifola frondosa*	MushroomScience	Yes
Immune Builder	*Agaricus blazei* *Cordyceps sinensis* *Coriolus versicolor* *Ganoderma lucidum* *Lentinula edodes* *Grifola frondosa*	MushroomScience	No
IMMUTOL	*Saccharomyces cerevisiae*	Biotec ASA	No
Glucagel T	Barley	GraceLinc	
Senseiro	*Agaricus blazei*	Sundory	Yes
Glucan #300	*Saccharomyces cerevisiae*	Transfer Point	No

Table 2.

Comparison of individual glucans				
Name	**Phagocytosis**	**CD expression**	**Il-2 production**	**Antibody formation**
Now Beta Glucan	++	+++	++	++
MaitakeGold 404	++	+++	++	++
Immune Builder	++	+	+	-
IMMUTOL	++	+	+	+
Glucagel T	-	-	+/-	+++
Senseiro	+	+	-	-
Glucan #300	+++	+++	+++	+++

have limited activity and β-glucans with a 1,3 configuration with additional branching at the position 0-6 of the 1-3 linked D-glucose residues have the highest immunostimulating activity.[15] Readers seeking additional reviews might see Kogan[16] or Vetvicka.[17] However, it is important to keep in mind that these reviews are oriented towards comparing results of numerous publications and none of them offers a face-to-face comparison of several glucans. At the same time, with the high number of individual glucans and huge differences in their biological activities, it is imperative to evaluate their biological properties before any suggestions for use of a particular glucan can be made.

In our paper, we compared seven commercially successful glucans, differing both in source (mushroom, yeast and barley) and solubility. At the same time, we used identical amounts of glucans from each sample. In the case of complex mixtures (such as Immune Builder), the total amount of used sample corresponded to the ratio of individual glucans.

As various glucans are well known to stimulate phagocytosis,[18] one of the first tests of the immunological characteristics of any glucan is phagocytosis. We used the 2-hydroxyethyl methacrylate particles, which have only a slight negative charge and thus do not nonspecifically adhere to the cell surface. This guarantees that only phagocytosing cells will engulf these particles and significantly lowers the chance of false negativity.[19] Our investigation showed that while most of the tested glucans stimulated phagocytosis of synthetic microspheres (with the exception of Glucagel T), the highest effects were obtained with Glucan #300.

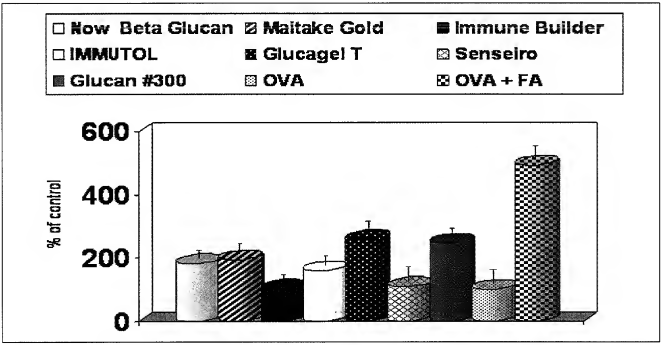
As some of the glucans are known to regulate the influx of cells into individual lymphatic organs,[8] we compared the effects of a single injection on expression of the basic membrane markers present on splenocytes. Only three glucans–Now Beta Glucan, Maitake Gold, and Glucan #300 – changed the number of CD4- and CD8-positive lymphocytes. No glucan significantly changed the percentage of B lymphocytes. The effects on CD4-positive cells corresponded to the previously found effects of Phycarine[8] or lentinan.[20]

In addition to the direct effect on various cells of the immune system, the immunostimulating action of β-glucans is caused by potentiation of a synthesis and release of several cytokines such as TNFα, IFNα, IL-1 and IL-2. This cytokine–stimulating activity is dependent on the triple helix conformation.[21] The only glucan without a trace of pro-inflammatory cytokine stimulation is PGG-glucan.[22] We focused on the stimulation of IL-2 production by spleen cells in vitro and found that whereas all glucans (with the exception of Senseiro) stimulated production of IL-2, only two of the samples (Maitake Gold and Glucan #300) showed stimulation comparable to the common stimulator Concanavalin A. The activity of the most active glucan was comparable to the previously published data.[9,23]

Glucans are usually considered stimulators or modulators of the cellular branch of immune reaction and very little attention has been focused on their potential effects on antibody response. We decided to take advantage of the recently published method of evaluating the use of glucan as an adjuvant.[24] Our results rather surprisingly showed that most of the tested glucans revealed some level of stimulation of antibody response, the strongest being Glucagel T and Glucan #300. In this case, however, the stimulation was always significantly lower than in the case of combining antigen and Freund's adjuvant.

Data presented in this study and summarized in Table 2 clearly demonstrated the differences in activities among individual types of glucans. Also, it is clear that individual glucans can be highly active in one particular part of immune reactions (e.g., Glucagel T on antibody production), and almost without any significant biological activity in other parts of defense reaction. Glucan #300 showed not only a broad range of action, but in all tested reactions (with

Figure 4.



Effects of two ip. injections of tested glucans on formation of antibodies against ovalbumin. Mice were injected twice (two weeks apart) and the serum was collected 7 days after last injection. Level of specific antibodies against ovalbumin was detected by ELISA. As positive control, Freund's adjuvant was used. *Represents significant differences between control (ovalbumin alone) and samples at $P \leq 0.05$ level.

the exception of the antibody formation where it was the second most active sample) was the biologically most relevant immunomodulator.

Several conclusions can be made: 1) Not all glucans are created equal; 2) some of the commercial glucans have surprisingly low activity; 3) most glucans differ in biological effects based on tested characteristics; and 4) for good results in immunomodulation, it is more imperative to find a glucan from a solid vendor who is able to back the claims with solid scientific data. Thinking about the biological source of glucan is much less important.

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